IDENTIFICATION OF NON FERMENTING GRAM NEGATIVE BACILLI FROM CLINICAL, ENVIRONMENTAL SAMPLES, THEIR ANTIMICROBIAL RESISTANCE AND DETECTION OF bla_{VIM}/bla_{IMP} GENES IN IMIPENEM RESISTANT ISOLATES

Dissertation submitted in partial fulfillment of the

Requirement for the award of the Degree of

M.D. MICROBIOLOGY (BRANCH IV)

CHENNAI MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE

IRUNGALUR, TRICHY- 621 105

Affiliated To

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY,

CHENNAI, TAMILNADU

APRIL– 2016

CERTIFICATE

This is to certify that the dissertation entitled, **"Identification Of Non Fermenting Gram Negative Bacilli From Clinical, Environmental Samples, their Antimicrobial Resistance and Detection Of blaVIM/blaIMP genes in Imipenem resistant isolates"** by **Dr.M.Nivitha**, Post graduate in Microbiology (2013-2016), is a bonafide research work carried out under our direct supervision and guidance and is submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai, for M.D. Degree Examination in Microbiology, Branch IV, to be held in March 2016.

Guide:

Dr. A. Uma, M.D
Professor and Head,
Department of Microbiology,
CMCH&RC.

Professor and Head:

Dr. A. Uma, M.D
Professor and Head,
Department of Microbiology,
Chennai Medical College Hospital
and Research Centre (SRM Group).

Dean : **Dr. P.G. Shankaranarayanan, M.D**
Chennai Medical College Hospital
and Research Centre (SRM Group)
Irungalur,
Thiruchirapalli-621 105.
Tamil Nadu.

DECLARATION

I solemnly declare that the dissertation titled **"Identification of Non Fermenting Gram Negative Bacilli from Clinical, Environmental Samples, their Antimicrobial Resistance and detection of blaVIM/blaIMP genes in Imipenem resistant isolates"** is done by me at Chennai Medical College Hospital and Research centre, Trichy.

The dissertation is submitted to The Tamil Nadu Dr.M.G.R Medical University towards the partial fulfillment of requirements for the award of M.D Degree (Branch IV) in Microbiology.

Place: Trichy

Date**:**

Dr.M.Nivitha,

Post Graduate Student,
M.D Microbiology,
Chennai Medical College Hospital
and Research Centre (SRM Group),
Irungalur,
Trichy.

CHENNAI MEDICAL COLLEGE HOSPITAL & RESEARCH CENTRE

IRUNGALUR, TRICHY – 621 105.
E.Mail : iec.cmchrc@gmail.com, Phone: 0431-3058661,3058817

INSTITUTIONAL ETHICS COMMITTEE CERTIFICATE.

Ref.No: CMCH&RC/IEC –No:59 /13.05.2015

Sub: Approval of research work related to / project of faculty- IEC – Issued-Reg.

The research proposal submitted by, **Dr.M.Nivitha,** Post Graduate ,Department of Microbiology, Chennai Medical College, was discussed and analyzed by the Institutional Ethics Committee of the CMCH&RC. The committee approved the research project subject to existing rules and regulations.

Title of the Research work/Project:
Identification of Non-Fermenting gram negative bacilli in clinical and environmental samples and their antibiogram and detection of blaVIM/blaIMP gene in imipenem resistant isolates.

a. She should abide to the Ethical aspects of the institutional Ethics Committee.
b. She should not deviate from the proposal submitted
c. She has to inform IEC if any deviation / modification whenever considered.
d. She should carry out the project within the stipulated period and if extension needed, She has to inform IEC
e .She should get appropriately designed informed consent subjects/patients of the study group.
f. She should not claim any monetary support from IEC
g. She should cooperate with the members of IEC while they visit / monitor the activities
h. She should entitle to make use of this approval letter for obtaining financial support from funding agencies by submitting his application through the Dean, CMCH&RC.
i. She is informed that she should submit the summary of the report to the IEC after completion of her project
k. **IEC Comments: Approved, Consent from study subjects to be obtained and kept in her custody for verification by IEC any time.**

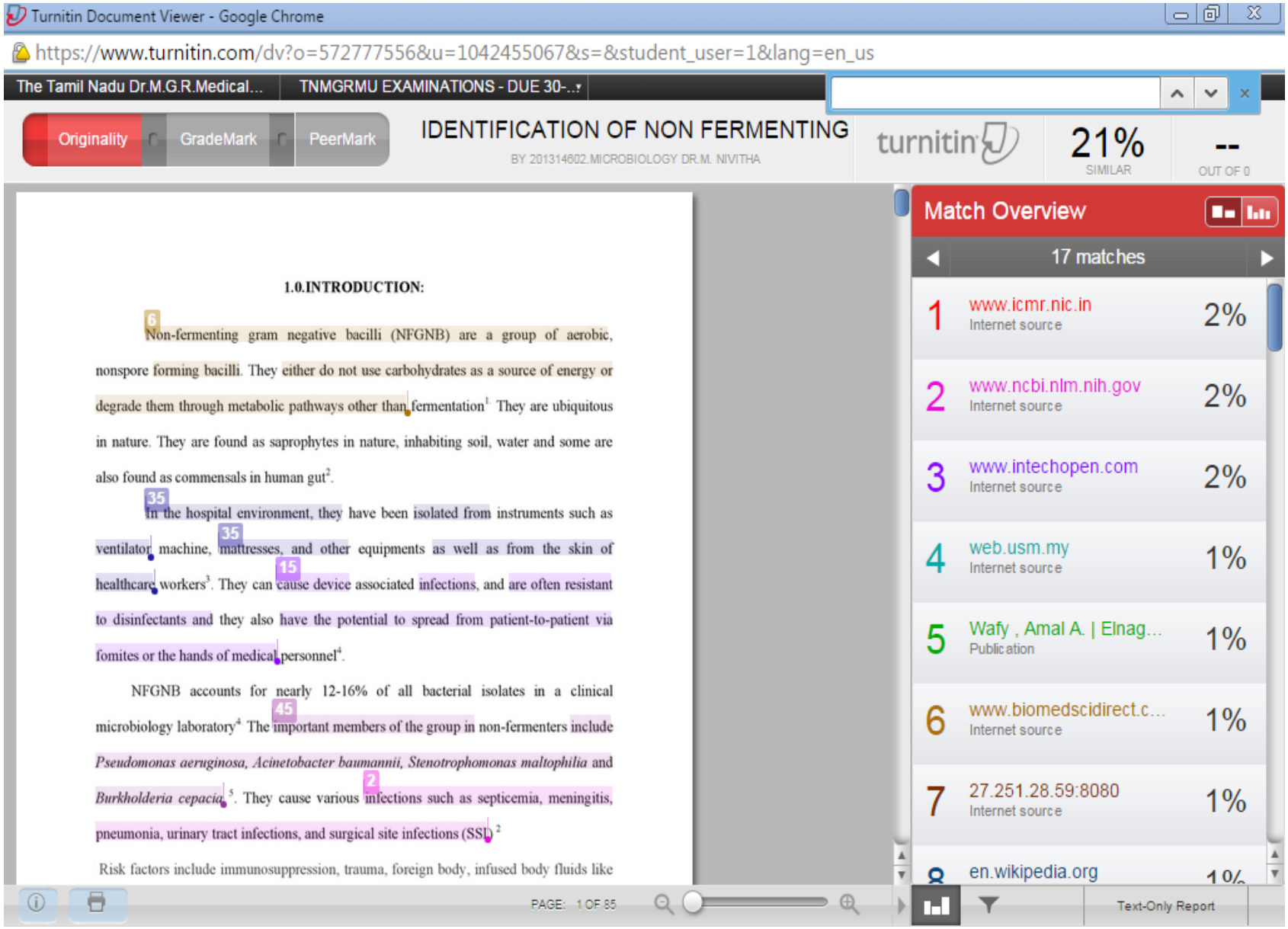
P. Rajen
Member Secretary
[Dr.P.Rajendran]

INSTITUTIONAL ETHICS COMMITTEE
Chennai Medical College Hospital & Research Centre
Irungalur, Trichy-621 105
Date.....

Member Secretary
INSTITUTIONAL ETHICS COMMITTEE

TO
Dr.M.Nivitha, Post Graduate ,Department of Microbiology, CMCH&RC, Trichy.
Copy to office

CHENNAI MEDICAL COLLEGE HOSPITAL & RESEARCH CENTRE


Turnitin Document Viewer - Google Chrome
https://www.turnitin.com/dv?o=572777556&u=1042455067&s=&student_user=1&lang=en_us
The Tamil Nadu Dr.M.G.R.Medical...
TNMGRMU EXAMINATIONS - DUE 30-...
Originality
GradeMark
PeerMark
IDENTIFICATION OF NON FERMENTING
BY 201314602.MICROBIOLOGY DR.M. NIVITHA
turnitin
21%
SIMILAR
--
OUT OF 0
1.0.INTRODUCTION:
Non-fermenting gram negative bacilli (NFGNB) are a group of aerobic, nonspore forming bacilli. They either do not use carbohydrates as a source of energy or degrade them through metabolic pathways other than fermentation[1]. They are ubiquitous in nature. They are found as saprophytes in nature, inhabiting soil, water and some are also found as commensals in human gut[2].
In the hospital environment, they have been isolated from instruments such as ventilator machine, mattresses, and other equipments as well as from the skin of healthcare workers[3]. They can cause device associated infections, and are often resistant to disinfectants and they also have the potential to spread from patient-to-patient via fomites or the hands of medical personnel[4].
NFGNB accounts for nearly 12-16% of all bacterial isolates in a clinical microbiology laboratory[4]. The important members of the group in non-fermenters include *Pseudomonas aeruginosa, Acinetobacter baumannii, Stenotrophomonas maltophilia* and *Burkholderia cepacia*[5]. They cause various infections such as septicemia, meningitis, pneumonia, urinary tract infections, and surgical site infections (SSI)[2]
Risk factors include immunosuppression, trauma, foreign body, infused body fluids like
PAGE: 1 OF 85
Match Overview
17 matches
1 www.icmr.nic.in Internet source 2%
2 www.ncbi.nlm.nih.gov Internet source 2%
3 www.intechopen.com Internet source 2%
4 web.usm.my Internet source 1%
5 Wafy , Amal A. | Elnag... Publication 1%
6 www.biomedscidirect.c... Internet source 1%
7 27.251.28.59:8080 Internet source 1%
8 en.wikipedia.org 1%
Text-Only Report

ACKNOWLEDGEMENT

I sincerely express my heartful gratitude to the Dean, Chennai Medical College Hospital and Research Centre, Trichy for all the facilities provided for the study.

I take this opportunity to express my profound gratitude to Dr .A.Uma, M.D., Professor and Head, Department of Microbiology, Chennai Medical College Hospital and Research Centre, whose kindness, guidance and constant encouragement enabled me to complete this study.

I am extremely thankful to Dr. P.Chitra Rajalakshmi M.D., Professor, Department of Microbiology for her guidance and support.

I wish to thank Dr.G,Vazhalavandal M.D., Dr.A.R.Susethira,M.D., Associate Professors, Department of Microbiology, Chennai Medical College Hospital and Research Centre, for their valuable guidance for the study.

I am highly obliged to Dr.R.Saraswathy,M.D., Dr.A.Anupriya,M.D., Dr.K.Lakshmi,M.D, Dr.J.Lalithambigai,M.D., Dr.Diego Edwin,M.D., Assistant Professors, Department of Microbiology, Chennai Medical College Hospital and Research Centre, for evincing keen interest, encouragement during the research period.

I wish to thank Mr.Vallab Ganesh,M.Sc.., Assistant Professor, Department of Microbiology, Chennai Medical College Hospital and Research Centre for his help and encouragement at the initial stage of my work.

Special thanks are due to my co-postgraduate Dr.J.Meera for lending a helping hand throughout the Study. I would also wish to thank my junior post-graduate colleagues for their help and support.

I would like to acknowledge the help rendered by all the technicians and non teaching staff in the department in conducting this study.

Last but not the least, I am indebted to my father, mother, sisters, friends and other family members for their moral support during the period of my study.

CONTENTS

TABLE OF FIGURES

CONTENTS OF TABLES

ABBREVIATIONS

NFGNB	- Non-fermenting gram negative bacilli
P.aeruginosa	- Pseudomonas aeruginosa
A.baumannnii	- Acinetobacter baumannii
F.meningosepticum	- Flavobacterium meningosepticum
BAP	- Blood agar plate
Mac	- Mac conky agar plate
CA	-Chocolate agar
MR	- Methyl Red
VP	- Voges proskaeur
TSI	- Triple sugar iron
EDS	- EDTA disk synergy test
ESBL	- Extended spectrum beta lactamse
MBL	- Metallo beta lactamase
IPM gene	-Imipenamase metallo betalactamase
VIM gene	- Verona imipenamase metallo beta lactamase
SPSS	- Statistical package for social sciences
SSI	-surgical site infections
CF	- cystic fibrosis

ABSTRACT

Introduction:

Non-fermenting gram negative bacilli (NFGNB) are a group of aerobic, nonspore forming bacilli. They are ubiquitous in nature, inhabiting soil, water and also present in the hospital environment. NFGNB accounts for nearly 12-16% of all bacterial isolates in a clinical microbiology laboratory. The important members are *Pseudomonas aeruginosa*, *Acinetobacter baumannii*. They cause various infections such as septicemia, meningitis, pneumonia, UTI, and surgical site infections. Risk factors include immunosuppression, trauma, foreign body, infused body fluids, indwelling catheters, prolonged hospital stay and using broad spectrum antibiotics

Materials and methods:

A hospital based cross-sectional descriptive study was undertaken between August 2014 to May 2015 to isolate NGFNB from various clinical samples received for culture and sensitivity in department of microbiology and environmental samples from ICUs and burns unit in tertiary care hospital, Trichy. The NFGNBs were identified using standard methods and antimicrobial susceptibility test was performed to determine the sensitivity pattern of the isolates. The isolates were screened for imipenem resistance and various phenotypic methods like combined disk method, EDS disk synergy test, MBL E- test were performed for identification of MBL genes. Genotypic detection of bla_{VIM} and bla_{IMP} genes was performed using multiplex PCR.

Results:

NFGNB was isolated most commonly from pus sample(52.3%). The commonest NFGNB to be isolated was *P.aeruginosa*(58%) followed by *A.baumannii*(20%). *P.aeruginosa* showed highest sensitivity to imipenem(86%) and *A.baumannii* showed highest sensitivity to Aztronam(80%).14% of *P.aeruginosa* were resistant to imipenem, and 57% of *A.baumannii* were resistant to imipenem. In phenotypic tests for MBL detection, 6, 8 and 10 isolates showed positivitiy for MBL gene by Combined disc test, EDS test, MBL E test respectively. Genotypic study showed that bla_{IMP} was present in 7 isolates bla_{VIM} gene 3, and in one isolate both the genes were present. In hospital environment *P.aeruginosa* was isolated in 5 sites and *A,baumannii* in 2 sites. All the isolates were sensitive strains.

CONCLUSION:

It is evident from the study that NFGNB are becoming resistant to carbapenems. To prevent the spread of the resistant bacteria, it is critically important to have strict antibiotic policies.

Key words: NFGNB, Carbapenem resistance, MBL, $bla_{IMP,}$ bla_{VIM} gene.

1.0.INTRODUCTION:

Non-fermenting gram negative bacilli (NFGNB) are a group of aerobic, nonspore forming bacilli. They either do not use carbohydrates as a source of energy or degrade them through metabolic pathways other than fermentation[1.] They are ubiquitous in nature. They are found as saprophytes in nature, inhabiting soil, water and some are also found as commensals in human gut[2].

In the hospital environment, they have been isolated from instruments such as ventilator machine, mattresses, and other equipments as well as from the skin of healthcare workers[3]. They can cause device associated infections, and are often resistant to disinfectants and they also have the potential to spread from patient-to-patient via fomites or the hands of medical personnel[4].

NFGNB accounts for nearly 12-16% of all bacterial isolates in a clinical microbiology laboratory[4.] The important members of the group in non-fermenters include *Pseudomonas aeruginosa, Acinetobacter baumannii, Stenotrophomonas maltophilia* and *Burkholderia cepacia* [5]. They cause various infections such as septicemia, meningitis, pneumonia, urinary tract infections, and surgical site infections (SSI) [2]

Risk factors include immunosuppression, trauma, foreign body, infused body fluids like saline irrigations, indwelling catheters, invasive diagnostic as well as therapeutic procedures, prolonged hospital stay and using broad spectrum antibiotics [6 7].

P.aeruginosa , Burkholderhia cepacia and Stenotrophomonas maltophilia are important pathogen of patients with cystic fibrosis (CF).They chronically infect the lung

of cystic fibrosis patient and once the infection has occurred, it is almost impossible to eradicate the organism. Development of multidrug resistance by non-fermenter isolate requires a number of different genetic events. It includes acquisition of mutations and/or horizontal transfer of antibiotic resistance genes.

P.aeruginosa has greater potential to become resistant to most antibiotics as it is clear from the fact that its genome contains the largest resistance island with more than 50 resistance genes. Mechanism for this antibiotic resistance is due to the production of antibiotic-degrading or antibiotic-inactivating enzymes, outer membrane proteins to expel the antibiotics and mutations that change the antibiotic targets [8].

Production of antibiotic-degrading enzymes such as extended-spectrum beta lactamases, AmpC cephalosporinases, carbapenemases like serine oxacillinases, metallo-beta-lactamases, OXA-type carbapenemases, aminoglycoside-modifying enzymes, among others non fermenting gram negative bacilli have been reported [9 10 11 12].

Carbapenem group of antibiotics are essential in the treatment of nosocomial gram negative infections, because of their wide spectrum of activity and stability to hydrolysis by most of the β-lactamases, including extended spectrum β-lactamases (ESBLs). Carbapenemases are classified mainly into two main molecular families: those with serine at their active site, called as serine carbapenemases, and those with at least one zinc atom at their active site, known as metallo-carbapenemases which are a subgroup of metallo-beta-lactamases (MBLs). Among the MBLs, the VIM and IMP types are the most clinically significant carbapenemases coded by bla_{VIM} and $blaI_{MP}$ genes. Nosocomial outbreaks of carbapenem-resistant *Pseudomonas aeruginosa* and

A*cinetobacter baumanii* because of metallo-β-lactamase (MBL) production have been reported from different regions[13 14 15].

The emergence of these Metalo beta Lactamases in gram negative bacilli is becoming a therapeutic challenge, because these enzymes have high hydrolytic activity resulting in degradation of higher generation cephalosporins. Therefore the treatment options are not available, or very expensive or toxic with poor outcome[16]. The genes responsible for the production of MBL may be chromosomally or plasmid mediated. As a result they pose a great threat of spread of resistance by gene transfer among the Gram negative bacteria. Therefore it is necessary to rapidly detect MBL production to modify the treatment and also to start an effective infection control measures to stop their dissemination.

Hence this study was undertaken to isolate and identify the various non fermenters from patients admitted in our tertiary care hospital and from environmental source as they are ubiquitously distributed and to study their antimicrobial susceptibility pattern and carry out phenotypic tests to identify carbapenemase producers and to identify the various health care associated infections they cause.

AIM AND OBJECTIVES

2.0 AIM OF THE STUDY

- To estimate the prevalence of nonfermenters in various clinical samples in a tertiary care hospital
- To assess frequency of isolation of non fermenters in hospital environments such as ICUs burns unit, etc.
- To study the antimicrobial susceptibility pattern of the isolates & to estimate the antibiotic resistance rate.
- To correlate the clinical sample & environmental isolates with the clinical infections
- To detect MBL by phenotypic and genotypic methods among imipenem resistant isolates.
- To create awareness on prevention of hospital acquired infection related to nonfermenters among hospital staff and judicious use of antibiotics.

REVIEW OF LITERATURE

3.0. REVIEW OF LITERATURE

The NFGNBs are a group of Gram negative bacilli or coccobacilli which do not make use of carbohydrate as a source of energy by fermentation and hence termed as 'Non-fermenters'. However, they may utilize carbohydrate by oxidative method .The NFGNBs have been defined as all aerobic Gram negative bacilli that shows abundant growth within 24 hrs on the surface of TSI medium but neither grows in nor acidifies the butt of the media[1].

3.1. HISTORICAL PERSPECTIVE:

Before the advent of modern medical microbiology there was evidence that *Pseudomonas aeruginosa* was a cause of serious wound and surgical infections as eloberated by Doggett. In 1850, Sedillot noted that there were blue-green discharges on surgical dressings that were associated with infection[17].

The pigment was extracted by Fordos in 1860 and a crystalline substance pyocyanin was isolated[18].

In 1862, Luke noted rod shaped microorganisms were associated with within the blue green pus. It was Gessard who isolated the organisms and designated them as Bacillus pyocyaneus in 1882[17].

Osler in 1925 thought that the organism to be more of a secondary or opportunistic invader of damaged tissue as opposed to primary cause of infection in healthy tissue. In 1960s, it has emerged as a major human pathogen because of its ability to cause infections in immunocompromised hosts.

The genus *Acinetobacter* has suffered a long history of taxonomic change. The first member of the group described was a soil organism by enrichment in a calcium-acetate-containing minimal medium isolated by Beijerinck in 1911, and named by him as *Micrococcus calcoaceticus*. The genus *Acinetobacter* was proposed by Brisou and Prevot in 1954 [17] [18].

In 1959, American bacteriologist Elizabeth O.King isolated the organism from a case of peadiatric meningitis and named it as Flavobacterium meaning 'yellow bacillus' in Latin and meningosepticum likewise means associated with meningitis and septicemia. In 1954, it was reclassified in the genus Chryseobacterium, and was named Chryseobacterium meningosepticum , chyseo meaning 'golden'[19]. In 2005, it was placed in the genus Elizabethkingia named after the original discoverer of *F.meingospticum* meaning "unable to move" was adopted in 1969.

3.2. HABITAT:

Non fermenters have their natural habitat in environment and hence serve as potential reservoir for human infections [1 20 21 22].

1. Water reservoirs prevalent in hospital humidifiers, nebulizers, waterbaths, disinfectants, irrigation solutions, distilled water lines, soaps, injectable drugs.
2. Implements, such as anesthetic equipment, forceps, thermometers that may be stored in disinfectant solutions, and mops, sponges and towels, contact lens solutions.
3. Most intertriginous parts of the skin and mucous membrane, toe webs, groin, axilla and antecubital fossa, throat, nasal mucosa, gastro intestinal tract.

They can be colonizers or cause wide spectrum of diseases. NFGNBs are known to colonize first and then they subsequently invade the otherwise normally sterile site through trauma. It has been noted that disruption of natural barriers as an important route of entry of infections.

3.3. METABOLISM:

3.3.1. BACTERIAL PHYSIOLOGY OF NON-FERMENTERS:

NFGNB refers to all aerobic gram negative rods that show abundant growth on the surface of Triple sugar iron agar or Kligler iron agar (KIA) but do not grow or acidify the butt of these media[1 23]. Most of the routinely isolated, medically important bacteria utilize carbohydrates by one or several different pathways. However, some bacteria belonging to NFGNB like *Moraxella* species do not use carbohydrates as a source of

energy. They derive energy from others sources such as amino acids, alcohols and organic acids [1] .

The differentiation between NFGNB and other fastidious organisms which do not ferment glucose is based on convention rather than well defined genotypic or phenotypic characters[1]. In order to gain a clear understanding of NFGNB, it is important to understand the different metabolic pathways utilized by these organisms.

3.3.2. Fermentative and oxidative metabolism [1 24 17]

The bacterial degradation of carbohydrates proceeds by several metabolic pathways in which hydrogen ions are successively transferred to compounds of higher redox potential, with the ultimate release of energy in the form of adenosine triphosphate (ATP). All six-, five-, and four- carbon carbohydrates are initially degraded to pyruvic acid, an initial intermediate. Glucose is the main carbohydrate source of the carbon for bacteria and degradation proceeds by three major pathways:

1. The Embden- Meyerhof- Parnas pathway
2. The Entner- Doudoroff pathway
3. The Warburg-Dickins/ The Hexose monophosphate pathway

Glucose is converted to pyruvic by a series of reactions in each of these three pathways. Bacteria use one or more of these pathways of glucose metabolism depending on the availability of oxygen and the presence of enzymes within the organism.

The Embden-Meyerhof- Parnas (EMP) pathway:

It is also called the glycolytic or anaerobic pathway because glucose is degraded without the use of oxygen. This has been called the fermentative pathway as so organic compounds serve as the final electron acceptors. End products formed from this pathway are the mixed acids, which account for the drop in the pH in the fermentation tests.

The Entner – Doudoroff (ED) Pathway:

This is called the aerobic pathway because oxygen is required for glycolysis. This has been classically called the oxidative pathway, because pyruvic acid transfers the electrons into Kreb's cycle, where they are linked to elemental oxygen to form water. Therefore, through this pathway, the carbohydrates are oxidatively metabolized, where oxygen is required as the terminal electron acceptor.

The acids formed in this pathway are extremely weak and hence cannot be detected by the routinely used tests. Many of NFGNB use this pathway of carbohydrate metabolism.

The Warburg–Dickens/ Hexose Monophosphate Pathway:

This pathway is a hybrid between the EMP and ED pathways. It is used mainly by facultatively anaerobic organisms. Mixed acids are formed as end products in this pathway also. Hence the organisms using this pathway give fermentative reaction in routinely used biochemical tests.

3.4. CURRENT CLASSIFICATION:

The NFGNB do not fit into a single family of well- characterized genera1. The correct taxonomic placement of many of the NFGNB is still unsolved. The major genera of gram negative bacilli have been classified into 15 families including *Alcaligenaceae, Alteromonadaceae, Brucellaceae, Burkholderiaceae, Flavobacteriaceae, Methylobacteriaceae, Morxellaceae, Oceanospirillaceae, Pseudomonadaceae, Rhizobiaceae, Sphingobacteriaceae, Sphingomonadaceae and Xanthomonaceae*[1].

The medically important non-fermenters can also be classified depending on the presence or absence of motility and the type of flagella they possess as follows [1].

Table 1: Classification of NFGNB based on motility and type of flagella

MOTILE WITH POLAR FLAGELLA
Family Pseudomonadaceae (rRNA group I) Genus *Pseudomonas*
Family Burkholderiaceae (rRNA group II) Genus *Burkholderia* Genus *Cupriavidus* Genus *Lautropia* Genus *Pandorea* Genus *Ralstonia*
Family Comamonadaceae (rRNA group III)

Genus *Comamonas* Genus *Acidovorax* Genus *Delftia*
Family Caulobacteriaceae (rRNA group IV) Genus *Brevundimonas*
Family Xanthomonadaceae (rRNA Group V) Genus *Stenotrophomonas*
Family Sphingomonadaceae Genus *Sphingomonas*
Family Oceanospirillaceae Genus *Balneatrix* Genus *Massilia* **Family Alteromonadaceae** Genus *Shewanella* Genus *Alishewanella*
Family Oxalobacteriaceae Genus *Herbaspirillum* Genus *Massilia*
Organisms with uncertain taxonomic positions

CDC Groups Ic, O-1, O-2, O-3, Vb- 3
MOTILE WITH PERITRICHOUS FLAGELLA
Family Alcaligenaceae Genus *Achromobacter* Genus *Alcaligenes* Genus *Bordetella* Genus *Kerstersia* Genus *Oligella*
Family Rhizobaceae Genus *Rhizobium*
Family Brucellaceae Genus *Ochrobactrum*
Family Halomonadaceae Genus *Halomonas*
NON MOTILE **OXIDASE POSITIVE**
Family Flavobacteriaceae Genus *Flavobacterium* Genus *Bergeyella* Genus *Chryseobacterium* Genus *Empedobacter* Genus *Myroides* Genus *Weeksella*

Family Sphingobacteriaceae Genus *Sphingobacterium* Genus *Pedobacter*
Family Moraxellaceae Genus *Moraxella* Genus *Psychrobacter*
Family Neisseriaceae Genus *Neisseria*
Family Alcaligenaceae Genus *Oligella*
NON MOTILE, **OXIDASE NEGATIVE**
Family Moraxellaceae Genus *Acinetobacter*
Organisms whose taxonomic position is uncertain CDC group NO-1 CDC group EO-5

3.5. CHARACTERISTICS OF EACH FAMIY:

3.5.1. MOTILE WITH POLAR FLAGELLA:

Pseudomonads:

This consists of the genus Pseudomonas and some closely related genera many of which were formerly placd under in the genus Pseudomonas, make up a group often referrd to as Pseudomonads.The common characteristics of pseudomonads are – they are motile by means of a polar flagella. They are slightly curved or straight Gram negative rods. Most of them are oxidase positive[17 18 25] .

Family *Pseudomonadaceae*:

This consists of the genus *Pseudomonas*. Currently there are 160 species of *Pseudomonas,* of which 12 are medically important. Two schemes have been developed for classifying the organisms under this family. Gilardi separated the pseudomonads into 7 groups on the basis of phenotypic characters: fluorescent, stutzeri, alcaligenes, pseudomallei, acidovorans, facilis-delafieldii and dimunita[26].

On the other hand, Palleroni separated the family into 5 ribosomal RNA groups using rRNA-DNA hybridization aid in identification, the species are divided into the following important groups[27].

Phenotypic classification of the pseudomonads (polar flagellated NFGNBs):

rRNA GROUP 1

Fluorescent group - *Pseudomonas aeruginosa*
- *Pseudomonas fluorescens*
- *Pseudomonas putida*

Stutzeri group - *Pseudomonas stutzeri*
Pseudomonas mendocina
CDC group Vb-3

Alkaligenes group - *Pseudomonas alcaligenes*
Pseudomonas pseudoalcaligenes

rRNA GROUP 2

Psuedomallei group - *Burkholderia mallei*
- *Burkholderia pseudomalei*
- *Burkholderia cepacia complex*
- *Burkholderia gladioli*
- *Pandorea species*
- *Ralstonia species*
- *Cupriavidus species*

rRNA GROUP 3

Weak oxidizer group – *Comamonas acidovarans*
Comamonas terrigena
Acidovorax delafieldii
Acidovorax facilis
Acidovorax temperans
CDC WO-1

rRNA GROUP IV

Dimunita group - *Brevindimonas dimunita*

- *Brevindimonas vesicularis*

rRNA GROUP V

-*Strenotrophomonas maltophilia*

Yellow Pigmented group

-*Pseudomonas luteola*

- *Pseudomonasoryzihabitans*

-*Sphingomonas paucimobilis*

H2S positive group

-*Shewanella putrefaciens*

- *Shewanella algae*

Halophilic group

-*Alishewanella fetalis*

-*Haolmonas venusta*

-CDC halophilic non fermener group I

FLUORESCENT GROUP:

The three main species in this group are *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* and *Pseudomonas putida*. They are characterized by the production of water soluble pyoverdin pigment which fluoresces white to blue-green under ultraviolet light at the wavelength of 400nm. This pigment is enhanced in media containing high phosphate concentration[28 29 30].

Of these, only *Pseudomonas aeruginosa* produces the blue-green pigment pyocyanin. These pigments are easily identified in nutrient agars and sensitivity test agars[31].

Table 2: Key characteristics of the Fluorescent group

TEST	P.AERUGINOSA	P.FLUORESCENS	P.PUTIDA
Pyocyanin	+	-	-
Pyoverdin	+	+	+
NO_3 reduction	V	V	-
Growth at 42^0C	+	+	+

***P.aeruginosa*:**

It is the most common *Pseudomonad* frequently encountered from clinical specimens. It is prevalent among patients with burn wounds, cystic fibrosis, organ transplants[33]. Infection occurs at the place where moisture tends to build up like tracheostomies, indwelling catheters and burn wounds. It causes urinary tract infections, nosocomial pneumonia, and also devastating keratitis and endophthalmitis[1 2].

Table 3: Virulence factors of *P.aeruginosa*: [1 33 34]:

Virulence factors	Human effects
Lipopolysaccharide endotoxin	Cascade of inflammatory events , septic shock
Mucopolysaccharide capsule	Adhesion to epithelial cells, barrier to antibiotics, increased viscosity to bronchial secretions
Pili	Act as adhesions

Proteases (alkaline & neural metalloproteinase)	Damage tissues (active on elastin, collagen, fibrin) Digestion of protecting proteins serving as host defenses
Neuraminidase	Enhances pilin mediated adherence
Phenazine pigment	Ciliary distruption
2 hemolysins : Phospholipase C glycolipid-rhamnolipid (heat stable)	Distruption of Phospholiids of cell membranes, hydrolysis of lung surfactant and ciliostatic action
Siderophores	Help growth in iron limited conditions. Generation of toxic oxygen free radicals
Exotoxin A	Causes tissue damage, inhibits phagocytes, inhibits protein synthesis
Exoenzyme S	Binding specificity for glycolipids
Cytotoxin : leucocidin	Cytopathic effects on leucocytes and alteration of phospholipids on cell membranes
Antibiotic inactivating enzymes	β lactamases, Aminoglcoside inactivating enzymes

Host factors in pathogenesis:

Because the major manifestations of *P.aeruginosa* infection are nosocomial infection, chronic lung infection in CF, and contact lens associated keratitis, it is clear that the primary determinant of virulence factor is the health status of the host. Healthy humans are generally highly resistant to *P.aeruginosa* infection and immunocompromised status of the host is an important risk factor. Although many factors contribute to the host susceptibility to *P.aeruginosa* infection, one important

factor for community-acquired and nosocomial *P.aeruginosa* infection appear to be neutropenia[18].

Innate immune resistance to *P.aeruginosa* infection starts with the anatomic barrier function of the skin and mucosa surfaces, and any disruption to the integrity of these barriers establishes a situation in which *P.aeruginosa* can become invasive. Typically problems are associated with burn wounds and use of intravenous or urinary catheters, and particularly use of endotracheal tubes[18].

In burned and wounded skin, *P.aeruginosa* grows to sufficient levels to elaborate a multitude of toxins. One of the important factors implicates in pathogenesis is elastase.

Soluble host factors present on mucosal surfaces that have been implicates in high level resistance to *P.aeruginosa* include complement proteins, lung surfactans, and similar members of collectin family[18].

IDENTIFICATION:

MORPHOLOGY AND STAINING:

It is a thin, motile Gram negative non sporing bacillus usually with a single polar flagellum [1 31].

CULTURAL CHARACTERISTICS:

It grows readily on ordinary media. In Nutrient agar it produces, six distinct colony types[31].

Type 1 – large, low convex, rough in appearance, and often oval with long axis in the line of the inoculum streak. (Most common type)

Type 2 – small, smooth, doomed

Type 3 – small and rough

Type 4 – small rugose

Type 5 – mucoid alginate producing type (seen in Cystic fibrosis patient)

Type 6 – dwarf colonies

On blood agar, it has a characteristic appearance. It appears as large gray colonies with spreading periphery .colonies often have a alligator skin appearance and exhibit a metallic sheen[1].

Cultures produces characteristic grape like smell of aminoacetophenone. It produces 4 distinct pigments, pyocyanin, fluorescin, pyorubin, pyomelanin. King, Ward, and Raney developed Pseudomonas agar P (King A medium) for enhancing the production of pyocyanin and pyorubin pigment, and Pseudomonas agar F (King B medium) for enhancing fluorescein pigment production[31 28].

SELECTIVE MEDIA:

Cetrimide agar with nalidixic acid, Irgasan containing media are commercially available for selective isolation of *P.aeruginosa* especially from environmental sites[31].

BIOCHEMICAL REACTIONS:

It is catalase, oxidase and citrate positive and indole negative, do not produce H_2S, MR,VP negative, utilizes glucose only oxidatively, forming only acid. Nitrates are reduced to nitrates and further to gaseous nitrogen. Arginine hydrolase test is positive[1 31].

TYPING METHODS:

The classification and typing of *P.aeruginosa* is essential for epidemiological purposes as it has become a very important nosocomial pathogen.

Biotyping, Resistance phenotyping, serotyping, pyocin typing, bacteriophage typing were used but they are now replaced by molecular typing methods like Plasmidotyping, DNA fingerprinting, Ribotyping and Pulsotyping (most reliable technique) [35].

TREATMENT:

Management of *P.aeruginosa* infections may not be easy as only a narrow spectrum of drugs is effective against this organism. The drugs that are commonly used to treat the infections are carboxypenicillins (Carbenicillin, Ticaricillin) the ureidopenicillins (Piperacillin), the anitpseudomonal cephalosporins (Ceftazidime), monobactums (Aztreonam), carbapenems (Imipenem and Meropenem) and aminoglycosides (Gentamicin, Tobramycin, Amikacin). *P.aeruginosa* has the capability of carrying multiresistance plasmids and this feature has led to the emergence of some *P.aeruginosa* strains that are resistant to all antibiotics[36 37]. Colistin is increasingly becoming the drug of choice for such pan-resistant organisms.

***P.fluorescens and P.putida* :**

These species are easily distinguished from *P.aeruginosa* by their inability to form pyocyanin or grow at 42^0C. Both species are psychrophilic and survive at refrigerator temperatures. Therefore they may multiply in stored blood and related

products and give rise to fatal reactions when injected intravenously [18]. They are isolated from hospital floors, sinks etc.

It causes infections like pneumonia, catheter-related blood stream infections, acute cholecystitis and cholangitis, tonsillitis, thrombophlebitis, septic arthritis and skin and soft tissue infections [38].

Stutzeri group.

This group includes *P. stutzeri, P. mendocina* and the unnamed CDC group Vb-3. These organisms are soil denitrifiers and can grow with ammonia as their sole source of nitrogen [1 39].

P. stutzeri has been recovered from variety of sources like soil, water, manure, sewage, straw, baby formula, hospital equipments, eye cosmetics and various clinical samples[42]. It is a rare human pathogen and has been associated with infections like otitis media, pneumonia, conjunctivitis, endodcarditis and meningitis in HIV- positive individuals[40].

Colonies appear dry and wrinkled on primary isolation, which pit or adhere to the agar plate. They may be buff or brown in color[39].

P.mendocina and CDC group Vb- 3 are very rarely isolated from clinical samples. *P.mendocina* has been isolated from a case of endocarditis following mitral valve replacement [41 42]. It produces smooth, buttery colonies. CDC group Vb-3 has been isolated from a case of multiple myeloma patient [43].

ALCALIGENES GROUP:

The organisms in this group include *P.alcaligens*, *P.pseudoalcaligenes*, and Pseudomonas species CDC group 1. They are asaccharoytic and weakly saccharolytic in OF glucose medium. They rarely case human infections like eye infections, empyema and one case of fatal endocarditis have been reported[44].

YELLOW PIGMENTED GROUP:

P.lutola and *P.oryzihabitans* are included in this group. *P.luteola* is motile by multitrichous polar flagella, oxidase negative. In Mac Conkey and blood agar, it produces yellow coloured colonies that are wrinkled and are adherent to the agar[1]. They rarely cause human infections and has been isolated from cases of meningitis, septicemia, peritonitis, osteomyelitis[45 46].

P.oryzyhabitans is also motile, oxidase negative. Like *P.luteola* they also form rough, wrinkled colonies. They can be differentiated from *P.luteola* by being negative for esculin hydrolysis and by having single polar flagellum. They appear to an emerging pathogen in peritonitis related to continuous ambulatory peritoneal dialysis[47].

Family Burkholderiaceae:

rRNA GROUP II

All species in this group belong to the genus *Burkhloderia*. They are differentiated from Pseudomonads group by exhibiting resistance to polymyxin B and colistin.

The most important species of the genus Burkholderia is *Burkholderia cepacia*, *Burkholderia pseudomallei* and *Burkholderia mallei.*

***Burkholderia cepacia*:**

It has emerged as opportunistic infections in patients with chronic granulomatous disease and cystic fibrosis[48]. It has been isolated from wet sources and wet surfaces, including detergent solutions, intravenous fluids, nebulizer solutions, mouth washes. Clinical infections include pneumonia, pneumonitis in patients receiving contaminated anaesthetics, urinary infection in patients receiving contaminated irrigation fluids, endocarditis due to contaminated heart valves.

Patients acquire infection from environment or via patient to patient. They cause skin and soft tissue infection in individuals with burns and surgical wounds and genitourinary tract infection following instrumentation[17].

Selective media with bacteriostatic dyes, antibiotics, or low pH have been described for selective isolation of *B.cepacia*. These include Pseudomonas cepacia medium (PCM), containing crystal violet, polymyxin B and ticarcillin [49],OFLB medium containing polymyxin B, bacitracin and lactose[50] and *Burkholderia cepacia* selective agar (BCSA) containing lactose, sucrose, polymyxin B, gentamicin, and vancomycin. Studies show that recovery of this organism in patients with cystic fibrosis is enhanced with their use[51].

Burkholderia mallei:

It is oxidase positive, aerobic and non motile. It causes glanders which is a highly communicable disease of horses and mules and recognized as an occupational risk for horse handlers, veterinarians and lab workers.

Human glanders can be acute or chronic, with mode of infection, inoculating dose and host factors determining the clinical course. Infection occurs either by inhalation or direct inhalation or direct inoculation[52]. They are resistant to beta lactam antibiotics including first and second generation cephalosporins. The antibiotics used include aminoglycosides, macrolides, carbapenems and doxycycline[52].

***Burkholderia pseudomallei*:**

Burkholderia pseudomallei cause meliodisis, a glanders like diseases in animals and humans. The bacterium is found in contaminated soil and water. Infections are acquired by contact with the organisms either by inhalation of dust or direct contact through breaks in the skin.

It is oxidase positive, motile with polar flagella, arginine dihydrolase and gelatin positive and produce acid in OF lactose. On staining it shows bipolar safety pin pattern [52] . This organism grows well on routine laboratory media and often produces wrinkled colonies with distinctive earthy odour [53].

Selective agars like Ashdown's selective agar (ASA) are used for isolation of the organism from non sterile sites[54].

An important feature of this disease is it produces latent infection that can reactivate years after primary exposure. Three forms of melidiosis has been described; Acute disease presenting as septicemia with metastatic lesion, subacute disease presenting as pneumonia, and chronic disease presenting as chronic cellulitis.

Treatment should be started early in the course of disease[1].

Burkholderia gladioli:

Burkholderia gladioli is one of the few pseudomonads which is oxidase negative. Rarely does it cause human infctions like chronic granulamatous infections, soft tissue infections, keratits and endophthalmitis in patients with diabetes mellitus.

rRNA GROUP III:

It consists of family Comamonadaceae

Acidovarans group:

This group consists of *Comamonas acidovarans*, *Comamonas testosterone* and *Comamonas terrigena.* All are motile with tuft polar flagella. Acid is not produced in OF glucose medium and thus these organisms are grouped among the alkaline *pseudomonads*[1].

Comamonas acidovarans was placed in new genus *Delftia. D.acidovarans* is the most common of this group to be isolated from clinical specimens. It has the unique feature of producing an orange indole reaction due to the production of anthranilic acid from tryptone[1].

Facilis-delafieldii group:

This group includes Acidovorax facilis, *Acidovorax delafieldii and Acidovorax temporans. acidovorax delafieldii and acidovorax temporans* have been isolated from clinical specimens.

rRNA GROUP IV:

All species in this group have transferred to new genus *Brevundimonas.* This group consists of *Brevundimonas diminuta* and *Brevundimonas vesicularis. Brevundimonas*

vesicularis is slow growing and produces dark yellow pigment. It has been isolated from dialysis fluid, oral abscess, scalp wound and in blood[1].

rRNA GROUP V:

GENUS STENOTROPHOMONAS:

It is the third most frequently encountered non fermenter[1]. (Koneman 2006)

They are gram negative rods, motile and susceptible to Polymyxin B. *Stenotrophomonas maltophilia*, is an emerging nosocomial pathogen. Most commonly present in environment (rivers, wells, sewage, and bottled water) and food stuffs like fish, milk, eggs. They are often recovered from respiratory tract. They have known to cause pneumonia, bacteremia, endocarditis, cholangitis, UTI, meningitis, wound infections, intra abdominal abscess, conjunctivitis, keratitis[24].

Important identification features:

-oxidase negative

-Oxidizes glucose and maltose

-DNase and lysine decarboxylase

On Blood agar, it appears rough, lavender gray with ammonium odour.

S. maltophiila is inherently resistant to commonly used antipseudomonal drugs. This favours colonization of this organism and increases the risk of infection. It is inherently susceptible to trimethoprim-sulfamethoxazole which has become the drug of choice in treating infections due to *S.maltophilia* [1].

Genus Sphingomonas:

S.paucimobilis is the most common species recovered from clinical infections. It is a gram negative motile rod which is oxidase positive. Isolates are strongly esculin hydrolysis and produces zone of inhibition around vancomycin disk (30µg) placed on BAP. They have been recovered from variety of clinical infections like septicemia, meningitis, wound infections and also from hospital environment[55].

Most of the strains are susceptible to tetracycline, chloramphenicol, trimethoprim-sulfamethoxazole and aminoglycosides.

GENUS SHEWANELLA – HYDROGEN SULFIDE PRODUCING GROUP:

This genus consists of around 22 species. Most important species are *S.putrefaciens*, and *S.algae*. *S.algae* is the one which is predominantly isolated from human infections and *S.putrefaciens* represents majority of non human isolates. *S.algae* requires NaCl for its growth.

They are easily distinguished from other groups because these are the only non fermenters which produce hydrogen sulfide in KIA and TSI[1]. (Koneman 2006) They have been isolated from skin ulcers, eye infections, ear infections, arthiritis, endocarditis, bacteremia[56].They are generally susceptible to most antimicrobial agents except penicillin, cephalothin.

GENUS ALISHEWANELLA – HALOPHILIC GROUP:

Alishewanella fecalis is a halophilic organism which requires Nacl for its growth. It does not produce hydrogen sulfide in TSI agar.

3.5.2 MOTILE WITH PERITRICHOUS FLAGELLA:

GENUS ALGALIGENS:

A.fecalis is the most frequently isolated species. It produces strong alkaline reaction in al carbohydrate media. It produces characteristic fruity odor (odor of green apples)[1].(Koneman 2006) It exists in soil and water and have been isolated from clinical infections like acute otitis media, urinary tract infections and bacteremia[57 58].

GENUS ACHROMOBACTER:

A.xyloxidans oxidizes OF glucose and xylose, hence the name. It has been isolated from blood, CSF, bronchial washings, urine, pus wound.

3.5.3 NON MOTILE, OXIDASE POSITIVE:

FAMILY FLAVOBACTERIACEAE:

Chryseobacterium species are long, thin and slightly curved GNB. *Chryseobacterium meningosepticum* colonies usually form dark, yellow pigments in culture, and fail to grow on MacConkey agar. They are non motile, produce positive catalase and oxidase reactions[1].

Ubiquitous in nature, they are inhabitants in soil and water. Frequent colonization of patients via contaminated medical devices has also been reported.

Chryseobacterium meningosepticum is a cause of neonatal meningitis, especially in premature infants during the first two weeks of life. Clusters of neonatal meningitis have been linked to many sources including contaminated saline solutions for flushing eyes, ventilators and sink drains. In adults it causes endocarditis, pneumonia, and septicemia.

Environmental studies show that they can survive in chlorine treated municipal waters, and colonizes sink and it is a potential reservoir for infection in hospital environment[59].

It has got a unusual antibiotic profile. It is intrinsically resistant to most of the antibiotics which is used to treat gram negative infections like aminoglycosides, β-lactam agents, Chloramphenicol, carbapenems but susceptible to agents used to treat gram positive bacteria like Rifampicin, Ciprofloxacin, Vancomycin, trimethoprim–sulfamethoxazole. So the choice of treatment is difficult. The disc diffusion methods are not reliable and broth microdilution is the preferred method for detection of antibiotic susceotibility. Although vancomycin has been used to treat infection some studies shows resistance to vancomycin and minocycline, trimethoprim-sulphamethoxazole and rifampicin may be used as alternative drugs[60].

GENUS SPHINGOBACTERIUM:

The genus *Sphingobacterium* is yellow pigmented, oxidase positive and non motile. *S.multivorum* and *S.spiritivorum* are the two important species mostly isolated from human infections.

GENUS MORAXELLA:

These are tiny non motile gram negative diplococci which are oxidase and caalse positive. The medically important species are *M.lacunata*, M.nonliquefaciens, M.catarrhalis.

Moraxella are normal flora on mucosal surfaces and are considered to have low pathogenic potential. They occur most frequently in respiratory tract, less commonly genital tract infection and rarely cause systemic infections.

Most of the strains are susceptible to penicillin, cephalosporins, tetracycline, quinolones and aminoglycosides[1].

3.5.4 NON MOTILE, OXIDASE NEGATIVE:

GENUS ACINETOBACTER:

It is the second most frequent non fermenter isolated from clinical laboratories. They are strictly aerobic, gram negative coccobacilli that are oxidase negative, non-motile, nitrate reduction test negative[1].Originally, the genus was placed under the family Neisseriaceae, but now it has been moved to family Moraxellaceae.

The important species belonging to this genus are *Acinetobacter baumannii*, *Acinetobacter lwoffii*, *Acinetobacter haemolyticus*, *Acinetobacter calcoaceticus* and *Acinetobacter junii.*[61 62].

Morphology and staining:

In gram stain, they appear as cocci or coccobacilli.

Cultural characteristics:

On Mac conkey agar, colonies show slightly pinkish tint. On blood agar, they are not pigmented.

Biochemical reactions:

They are cytochrome oxidase negative, citrate positive, non motile, oxidizes glucose rapidly with the production of acid, oxidizes 10% lactose rapidly with the production of acid, and negative for nitrate reduction[31].

Table 4: Identification of *Acinetobacter* species:

TEST	*A.baumannii*	*A.lwoffii*
OF Glucose	Saccharolytic	Asaccharolytic
Arginine dihydrolase	Positive	Negative
Growth at 42^0C	Positive	Negative
10% Lactose	Positive	Negative
Chloramphenicol sensitivity	Resistant	Sensitive

Acinetobacter tend to be resistant to wide range of antibiotics, although one species, *A.lwoffii*, tends to be more sensitive to others. They are almost universally resistant to ampicillin, cephalothin, and most srains and are resistant to chloramphenicol. In recent years there has been increased resistance towards aminoglycoside resistance and carbapenem resistant strains in nosocomial outbreaks[63].

Treatment:

For serious infections combined treatment with aminoglycoside and ticarcillin or pipericillin is synergistic and effective. The only antimicrobial agent which is proved active against MDR *Acinetobacter* is colistin [1].

3.6. ANTIBIOTIC RESISTANCE MECHANISMS:

Antibiotic resistance is a international problem of foremost importance [64]. Isolations of multi-drug –resistant, extensively drug resistant or even pan drug resistant gram negative pathogens are causing major therapeutic problems and at the same time are posing infection control issues in many health care centres. This results in increased morbidity and mortality of the patients, increased length of hospital stay and higher hospital costs [65].

P.aeruginosa is a opportunistic nosocomial pathogen. They are responsible for variety of infections and show high rates of resistance to antibiotics. This is due o the fact that its genome is among the largest in the bacterial world allowing for larger genetic capacity and high adaptableness to environmental changes. It has 5567 genes encoded in 6.26 Mbp of DNA [8 70]. This largest genome is further enriched with addition of genes acquired by transferable genetic elements is a major contributing factor for development of resistance against all known antibiotics[70].

Three classes of antimicrobial resistance have been described.

1. Intrinsic resistance
2. Acquired resistance
3. Genetic resistance

INHERENT RESISTANCE [66]:

Inherent resistance to antimicrobial agents occurs through variety of mechanisms.

1) Decreased outer membrane permeability
2) Efflux systems
3) Synthesis of antibiotic inactivating enzymes

Outer membrane permeability:

Outer membrane of GNB is a semipermeable barrier which blocks the uptake of antibiotics and soluble molecules to pass through it. Sequential analysis of genes have identified 163 predicted outer membrane proteins which have grouped into three families of porins. The role of these porins is transport of sugars, aminoacids, divalent cations. Certain hydrophilic antibiotics like beta lactam antibiotics, aminoglycosides, fluoroquinolones also transverse the outer membrane through porins. Aminoglycosides and colistin ineract with the lipopolysaccharides of the outer membrane so as to pass through the membrane. Beta lactam antibiotics and quinolones disseminate through some porin channels.

The outer membrane of *P. aeruginosa* is only 8% as permeable as the outer membrane of *Escherichia coli*.

Most bacteria have lot of general porins and a relatively few specific porins. But *P.aeruginosa* express mainly specific porins. Loss of certain specific porin channels lead to reduced susceptibility of the antibiotics[67].

The OprD porin of *P.aeruginosa* is a substrate-specific porin permits the diffusion of basic amino acids, and carbapenems into the cell and loss of OprD porin from the outer membrane notably reduces the susceptibility of the organism to available carbapenems[19].

The Porins of *B.cepacia* and *Acinetobacter sp* have small porin channels [67 68]. (Gotoh 1994; Sato 1991) The porin size of *S.maltophila* is same as that of *E.coli* but in low copy numbers[70].

Efflux systems [37]:

P.aeruginosa posses a number of efflux pumps that actively pump the antibiotic out of the cell. This mechanism has been implicated in resistance of the organism to tetracycline, quinolone and beta lactams.

They consists of three proteins:

1) protein transporter of cytoplasmic membrane

2) periplasmic connective protein ,

3) an outer membrane porin.

These efflux system expels most of the antibiotics except polymyxins[71].

Antibiotic inactivating enzymes:

All strains of *P.aeruginosa* produce a chromosomally mediated AmpC-type beta lactamases and few plasmid mediated beta lactamases. Normally the chromosomal AmpC enzyme is in a repressed state but it can be strongly induced by first generation cephalosporins, cefoxitin and ampicillin. These antibiotics are liable to these enzymes as

well as strong inducers. Third generation cephalosporins and ureidopenicillins are also liable to the enzyme but do not induce its synthesis. So they are active as long as the enzyme is not induced. However stably derepressed mutants that produce AmpC copiously arise spontaneously and can be selected in vivo during cephalosporin or ureidopenicillin therapy. These mutants are resistant to all beta lactams except to imipenem and sometimes carbenicillin.

A. baumannii innately produces an AmpC-type cephalosporinase. They are also known as *Acinetobacter*-derived cephalosporinases (ADCs).In *A. baumannii* inducible AmpC expression does not occur unlike other gram-negative organisms[72]. Cefepime and carbapenems are resistant to the hydrolysis caused by these enzymes.

The main reason for the resistance of *A.baumannii* to carbapenem dugs is class D (OXA) carbapenemases . *Acinetobacter* strains also express Ambler class B MBLs, such as IMP, VIM, and SIM-1. They show high level of resistance to carbapenems and to other beta-lactam antibiotics except Aztreonam[73].

Extended-spectrum beta-lactamases from the Ambler class A group have also been described for *A. baumannii.* [74 75]

S. maltophilia has an inducible, chromosomally encoded class D beta lactamase. They efficiently hydrolyze imipenem and is a Zinc metallo enzyme rather than a serine metallo enzyme.

ACQUIRED RESISTANCE:

This may be due to mutation or attainment of resistance mechanism via horizontal gene transfer and can take place during improper antibiotic usage [20].

Mutation leads to over expression of endogenous beta lactamases or efflux pumps, target site modifications, and reduced expression of specific porins.

Mutational derepression of AmpC enzyme is one of the frequent mechanisms of beta-lactams. AmpC enzymes are not carbapenamases, but they posses low potential of carbapenem hydrolysis and their overproduction along with efflux pumps, overexpression and/or diminishes outer membrane permeability also may lead to carbapenem resistance.

Acquired beta lactamases are encoded by genes which are situated in transferable genetic elements such as plasmids or transposons frequently on integrons and also on mobile insertion sequences called ISCR elements. Occasionally resistance genes for other antibiotic classes are also located in the same integrons contributing to multi drug resistant phenotype [20].

Carbapenemases are of clinical importance because they inactivate carbapenem along with other beta lactam antibiotics. The class B carbapnemasess that bear Zn^{2+} in their active center are most common and are called metallo-betalactamases(MBLs.). They hydrolyse all beta lactams except the monobactam, aztreonam and are the most important cause of high level carbapenem resistance [13].

The activity of class B MBLs are not inhibited by clavulanic acid and tazobactam, but they are inacivated by bivalent ionic chelators,[76] e.g. EDTA. IMP, VIM, SPM, AIM, NDM-1 and GIM type MBLs were identified in *P. aeruginosa.*

The first carbapenemase to be confirmed *in P. aeruginosa* was IMP-1 in Japan from carbapenem resistant clinical isolates from 1992 – 1994 [77]. VIM-1 carbapenamases

are found in nosocomial *P.aeruginosa* strains in Italy in 1997 [78]. GIM, AIM and NDM producing *P.aeruginosa* are reported in Germany, Australia and Serbia respectively [76,79].

RESISTANCE TO FLUOROQUINOLONES:

Two mechanisms which leads to fluoroquinolone resistance in *P.aeruginosa* are Sstructural alteration in target enzymes and active efflux system.

DNA gyrase (GyrA and GyrB) is the primary target of fluoroquinolones in *P.aeruginosa*. This is due to point mutation in gyrA/gyrB genes which results in synthesis of modified topoisomerase II with reduced binding affinity to quinolone molecules. Modifications of a secondary target (topoisomerase IV) occur as a result of point mutations in parC and parE genes encoding ParC and ParE enzyme subunits, respectively [80 81].

In *Acinetobacter* strains resistance to flouroquinolones is mediated by mutation in gyrA and DNA topoisomerase mutations [82].

RESISTANCE TO AMINOGLYCOSIDES:[83]

Three different mechanisms are involved in the aminoglycoside resistance.

1) Acetylation by aminoglycoside acetyltransferases (AACs)
2) Adenylation by aminoglycoside nucleotidyltransferases (ANTs)
3) Phoshphorylation by aminoglycoside phosphotidyl transferases. (APHs)

Genes coding for AMEs are found on integrons.

Approaches to Overcoming Resistance:

1. combination therapy
2. using of antibiotic with an agent which overcome the antibiotic resistance mechanism

3. Boosting of patients specific and non specific defence mechanism[8]

3.7. IDENTIFICATION OF NFGNB:[1]

For the routine identification of NFGNB, there are different methods available which can be divided into:

A. Conventional tests

B. Commercial kit systems

Conventional tests:[1]

Different schemes are available for identification of NFGNB. The scheme for identification of the NFGNB depends on personal preference, past experience and the availability of the culture media required for identification. The schemes available are:

1. **Weyant, Giraldi and Pickett Identification schemes**

These systems have largest databases available for identification. They are based on the identification charts originally derived by Elizabeth King at the CDC.

2. **CDC scheme – Weyant and associates.**

This scheme consists of

a. An identification key for gram-negative aerobes

b. A set of 12 identification tables

c. A numerical code book by which derived biotype numbers can be linked to species names

They also include procedures and media formulas for all the biochemical tests cited in the identification manual. The main disadvantage of this system is that it is too voluminous and simplification of the system has become essential for using it routinely

3. The Giraldi scheme:

This is based on two fundamental principles:

a. The media and the tests are readily available in most clinical laboratories

b. Identification of most clinical isolates can be made in two stages – the first through the use of a primary battery of media and reactions that are sufficient in majority of the cases. A second set of reactions is then used, if identification using the primary set of reactions is not made.

This system is also very complicated to be used routinely. However, the recent modification to this scheme is devised for the small and medium-sized laboratories and is simpler and more practical than the original scheme.

4. The Pickett scheme

The highlight of this scheme is that, it was designed to rapidly identify two of the most frequently isolated nonfermenters, namely *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. If the isolates are other than these two common organisms, different tables are available for further identification, which are similar to the Weyant-CDC scheme.

Pratical approach to Identification of NFGNB

This approach is designed by P.C. Schreckenberger. This scheme divides the clinically important NFGNB into four functional groups, based on motility and the ability to produce cytochrome oxidase. Further tests are then carried out and the organism can be identified upto species level.

Advantages of this scheme are that

a. This scheme is simple compared to other available schemes

b. >95% of the NFGNB can be identified.

c. Routine media are sufficient for isolation and identification

Computer- aided schemes

DOS and Windows based schemes are available. With the results of the biochemical tests, NFGNB can be identified using databases.

Commercial kit based systems

Commonly used systems for the identification of NFGNB include :

a. Oxi/Ferm Tube (Becton Dickinson Microbiology Systems)

b. API 20E (BioMerieux)

c. API 20NE (Biomerieux)

d. Remel Uni-N/F System (Remel)

e. Crystal Enteric / Nonfermenter System (Becton Dickinson Microbiology Systems)

f. RapID NF Plus (Remel)

g. Biolog system (Biolog)

Advantages of these packaged systems are:

1. They are convenient to use,

2. Have a long shelf life

3. The need for fresh media and reagents is eliminated.

4. They also provide standardized techniques that are accurate and give reproducible results equal to or better than conventional procedures.

The problems in the use to these systems include:

1. The organisms that exhibit weak or delayed biochemical activity produce false-negative reactions

2. Many of these systems are designed inadequately, for cultivation of certain NFGNB

3. Inclusion of some tests that is not applicable to identification of NFGNB

3.8. TESTS FOR MBL CARBAPENEMASES: [84 85]

3.8.1PHENOTYPIC TESTS:

3.8.1.1 MBL SCREENING:

Isolates resistant to either Imipenem or Meropenem or Ceftazidime are considered screening positive and subjected to confirmatory tests. Various confirmatory tests have been described-

1. Imipenem- EDTA combined disc test
2. Imipenem – EDTA double disc synergy test

3. EDTA disc potentiation test

4. Modified Hodge test

5. MBL E-test

6. Carba NP test

3.8.1.2. Imipenem- EDTA combined disc test:[86 87]

Briefly, an overnight culture of an imipenem resistant clinical isolate was diluted with peptone water to 10^5 CFU/mL and spread on Mueller-Hinton (MH) agar plate using cotton swab. Two IPM disks were placed on the surface of the agar at a distance of 4-5 cm from each other. 5 µL of 750 µg/mL EDTA solution was then added to one of the IPM disks. The zone of inhibition displayed around the IPM and the IPM-EDTA disks were compared after 14 to 16 hrs of incubation at 37^0C

The difference of ≥7 mm between the inhibition zone diameter of the IPM-EDTA disk and that of IPM only disk was considered to be a positive for the presence of MBLs. [88].

3.8.1.3. Imipenem EDTA disk synergy (EDS) test [89]:

EDTA disk synergy (EDS) test is done by simultaneously testing two different β-lactams (imipenem and ceftazidime), for detection of metallo-β-lactamases in the imipenem resistant isolates.

A 0.5 M EDTA solution was prepared by dissolving 186.1 g of disodium EDTA $2H_2O$ in 1,000 ml of distilled water. The pH was adjusted to 8.0 by using Sodium hydroxide and was sterilized by autoclaving.

An overnight liquid culture of the test isolate is adjusted to a turbidity of 0.5 McFarland standard and spread on the surface of a MHA plate. A 10 µg imipenem disk and a 30 µg ceftazidime disk is placed on the agar. A blank disk (6 mm in diameter, Whatmann filter paper no. 1) was kept on the inner surface of the lid of the MHA plate and 10 µl of 0.5 M EDTA is added to it. This EDTA disk was then transferred to the surface of the agar and was kept 10 mm edge-to edge apart from the imipenem or ceftazidime disk. After incubating overnight at 37^oC, the presence of an expanded growth inhibition zone between the two disks is interpreted as positive for MBL production

3.8.1.4. EDTA disc potentiation using Ceftazidime, Ceftriaxone, Cefepime and Cefotaxime:

The test organism is inoculated on to plates with Mueller Hinton agar as described for the standard disc diffusion test. A blank filter paper disc is placed in the centre of the plate and the following discs: Ceftazidime (30 µg), Ceftriaxone (30 µg), Cefotaxime (30 µg), Cefepime (30 µg) are placed 25mm centre to centre from the blank disc. 10 µl of 0.5 M EDTA solution is added to the blank disc and the plate is incubated overnight at 35ºC. Enhancement of the zone of inhibition in the area between the EDTA disc and any one of the four discs in comparison with the zone of inhibition on the far side of the drug is interpreted as a positive result [90].

Modified HODGE Test: [91]

The meropenem resistant strains were subjected to modified Hodge test for detecting carbapenemases. An overnight culture suspension of *Escherichia coli* ATCC 25922 adjusted to 0.5 McFarland standard was inoculated using a sterile cotton swab on the surface of a Mueller-Hinton agar (MHA). After drying, 10 μg meropenem disk was placed at the center of the plate and the test strain was streaked from the edge of the disk to the periphery of the plate in four different directions. The plate was incubated overnight at 37^0C. The presence of a 'cloverleaf shaped' zone of inhibition due to carbapenemase production by the test strain was considered as positive.

MBL E-test [92 93]**:**

An overnight culture of an clinical isolate was diluted in peptone water to a turbidity of a 0.5 McFarland standard. A cotton swab was then used to transfer the inoculum onto a MH agar plate. Once dried, an E-test MBL strip (Himedia , Mumbai, India) was applied onto the plate which was then incubated at 37°C for 16 to 18 hrs to detect the presence of metallo enzymes.

Etest MBL strips contain increasing concentrations of imipenem (IP) on one end of the strip and imipenem overlaid with EDTA (IPI) on the other. The EDTA chelates the zinc ions required by MBLs to catalyse the hydrolysis of imipenem and meropenem, thereby inhibiting MBL activity. A reduction in the imipenem MIC in the presence of

EDTA of greater than or equal to eight-fold (IP/IPI ≥ 8) is interpreted as indicating MBL activity.

Carba NP test: [94 95 96]

The Carba NP test is a novel phenotypic method developed for carbapenemase detection. It is based on *in vitro* hydrolysis of imipenem by a bacterial lysate, which is detected by changes in pH values using the indicator phenol red (red to yellow/orange). It was reported to be 100% sensitive and specific for *Enterobacteriaceae* and 100% specific and 94.4% sensitive for *Pseudomonas* spp. harboring carbapenemases.

This test is based on biochemical detection of the hydrolysis of the beta-lactam ring of a carbapenem, imipenem, followed by change in the colour of a pH indicator. This test was performed on isolates grown on Mueller-Hinton agar plates at 37°C for 18 to 22 h . Isolated colonies are mixed with a solution of Imipenem and phenol red indicator solution and incubated at 37°C for a maximum of 2 h. If Carbapenemase producing organisms are present the Carbapenemase will hydrolyse the Imipenem, resulting in a decrease in pH. This is detected by a colour change of phenol red solution to yellow-orange.

MATERIALS AND METHODS

4.0 MATERIALS AND METHODS

4.1.1 Source of data

NFGNB isolated from heterogenous clinical samples like urine, blood, pus, sputum, pleural fluid, peritoneal fluid, blood and CSF received for culture and sensitivity in department of microbiology and environmental samples from various ICUs and burns unit in chennai medical college hospital and research centre, Trichy were included in the study.

4.1.2 Duration of data collection:

10 months (August 2014 to May 2015)

4.1.3 Sample size:

172 Non repetitive non fermenter isolates

4.1.4 Inclusion criteria:

1. Isolates from all clinical samples which were identified as Non-fermenting Gram negative bacilli as per recommended standard methods.

4.1.5 Exclusion criteria:

1. Mixed growth of > 3 types (probably contaminated sample)
2. Isolates from improperly collected samples.

4.1.6 Study design:

Hospital based Cross sectional descriptive study.

4.1.7 Data Collection:

All data were entered into a Data Collection proforma Sheet and were entered into SPSS data sheet.

4.1.8. Satistical Methods:

Statistical analysis were done using SPSS software version 22.0

4.2. Ethical committee approval:

Institutional Ethical committee approval was obtained before the start of the dissertation and the approval certificate is enclosed.

4.3. COLLECTION OF VARIOUS SAMPLES

4.3.1. RESPIRATORY TRACT SAMPLES:

Sputum sample was collected in a wide neck dry sterile container after proper instruction to the patient. Using the inoculation loop purulent portion of the sputum is transferred to the slide and a thin smear was made. The smear was then air dried, fixed and stained by the gram technique. Samples were then microscopically examined to assess contamination with upper respiratory tract secretions during collection. The sample is considered to be minimally contaminated if 25 pus cells and <10 epithelial cells/hpf.

Significance was laid to inflammatory cell response and predominance of bacteria in the pus cells in the gram stain preparation.

The endotracheal tube tips were received in sterile containers. The tips including the bore were washed with ~0.5ml sterile peptone water. This was vortexed thoroughly and the resulting suspension was used to inoculate the plate for culture.

All these respiratory samples were inoculated on sheep blood agar (BA), Mac Conkey Agar (MA) and Chocolate Agar (CA). All plates were incubated overnight at 37°C and observed for growth up to 48 hrs.

4.3.2. PUS & WOUND SWABS:

Using a sterile technique, pus was aspirated with syringe and needle or collected from a drainage tube preferably up to 5 ml and transferred to a leak-proof sterile container.

For wound swab, the area was wiped with sterile normal saline or 70% alcohol and the swab was taken along the leading edge of the wound. Two swabs are taken. One swab was used for culture and other for direct smear examination by Gram's stain.

These samples were inoculated on BA, MA, which were incubated overnight at 37^0C and observed for growth for 48 hrs.

4.3.3. URINE:

After washing their hands with soap and water, the female patient was instructed to cleanse the area around the urethral opening with clean water, dry the area with a sterile gauze pad, and collect the urine with the labia held apart. The same was done for the male patients after retracting the foreskin.

Clean catch midstream urine is collected in a sterile, dry, leak proof, transparent screw capped container. In case of indwelling catheters, the collection port was disinfected and 5 to 10ml of urine was aspirated with syringe and needle. The urine was processed within 2 hrs.

The gross appearance of the urine was noted. Wet film examination of the urine was done and the pus cells and bacteria were looked for.

A standard inoculation loop of diameter 4mm which delivers 10µl of urine was streaked in NA, Mac, and BA plates and incubated at 37^0c and observed for growth for 24 hours. Only those isolates found significant in semi quantitative culture of urine were included in the study.

4.3.4. BLOOD:

A brain heart infusion broth was used for blood culture. The bottle was examined daily for turbidity and subculture was made at regular intervals on to BA, MA and any growth was processed further for identification. NFGNB grown in blood culture were included if they grew in first two days of incubation of blood culture bottles.

4.3.5. ENVIRONMENTAL SAMPLES:

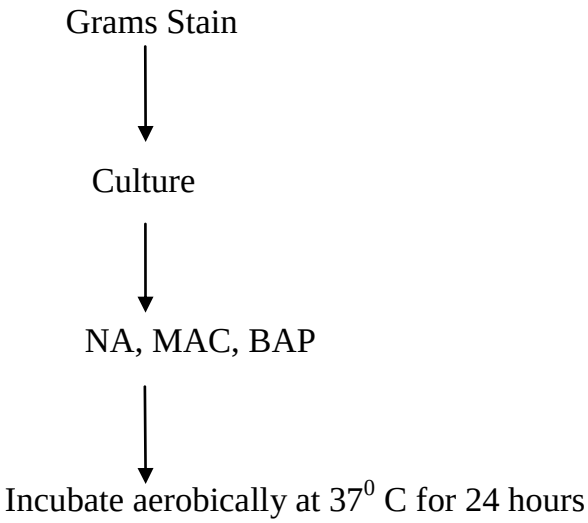
Samples from RICU, IMCU, Post operative ICU, PICU, Burns unit were taken. Samples from Staff hands, bed rails, wash basin sink, disinfectant solution, saline, nebulizer, oxygen mask, ambu mask, door handle were taken using saline moistened

sterile swabs. Tap water was collected in a sterile bottle after cleaning the mouth of the tap and allowing some water to flow out. The swabs were kept in nutrient broth overnight and inoculated in selective media for *Pseudomonas aeruginosa* and *Acinetobacter*, Pseudomonas isolation agar (Himedia Mumbai) and Leeds acinetobacter media (Himedia Mumbai) respectively.

Samples were collected under aseptic precaution and transported to the lab.

4.4. PROCESSING OF SAMPLES:

The study is conducted as follows:



Non lactose fermenting colonies were inoculated on Triple Sugar Iron agar (TSI) medium. .

4.5. IDENTIFICATION:

The most commonly isolated nonfermenters i.e., *P.aeruginosa, A.baumannii* and *S.maltophila* were identified by the following characteristics: [1]

4.5.1 IDENIFICATION OF PSEUDOMONAS AERUGINOSA:

Pseudomonas aeruginosa was identified by the presence of:

1. Large flat non-lactose fermenting colonies on MA
2. Large, flat, sometimes ß- haemolytic colonies on BA
3. Grape-like odour
4. Production of pyocyanin, pyoverdin, pyorubin pigment on NA, BA
5. Oxidase test positive.
6. Positive for motility
7. Growth at 42^0C

4.5.2. IDENIFICATION OF ACINETOBACTER BAUMANNII:

Acinetobacter baumannii was identified by following characteristics:[1]

1. Coccoid or coccobacillary appearance on Gram stain
2. Medium sized, smooth, convex colonies with a slightly pinkish tint on MA
3. Oxidase test negative
4. Rapid utilization of 10% lactose with acid production
5. Growth at 42^0C
6. Non- motile

4.5.3. IDENIFICATION OF STENOTROPHOMONAS MALTOPHILA:

Stenotrophomonas maltophila was identified by:[1]

1. Oxidase test negative
2. Production of acid in OF maltose
3. Lysine decarboxylase positive

If any other organisms were isolated other than the above, they were further identified based on the scheme of identification given by P.C. Schreckenberger. The organisms are grouped into four major groups based on Oxidase test and motility.

Further identification was done by using the following tests:

1. Hugh Leifson's Oxidative Fermentative test
2. Nitrate test
3. Indole test
4. Citrate utilisation test
5. Urease test
6. Triple Sugar Iron (TSI) agar
7. Arginine dihydrolase test
8. Lysine decarboxylase
9. OF sugars of mannitol, lactose and dextrose

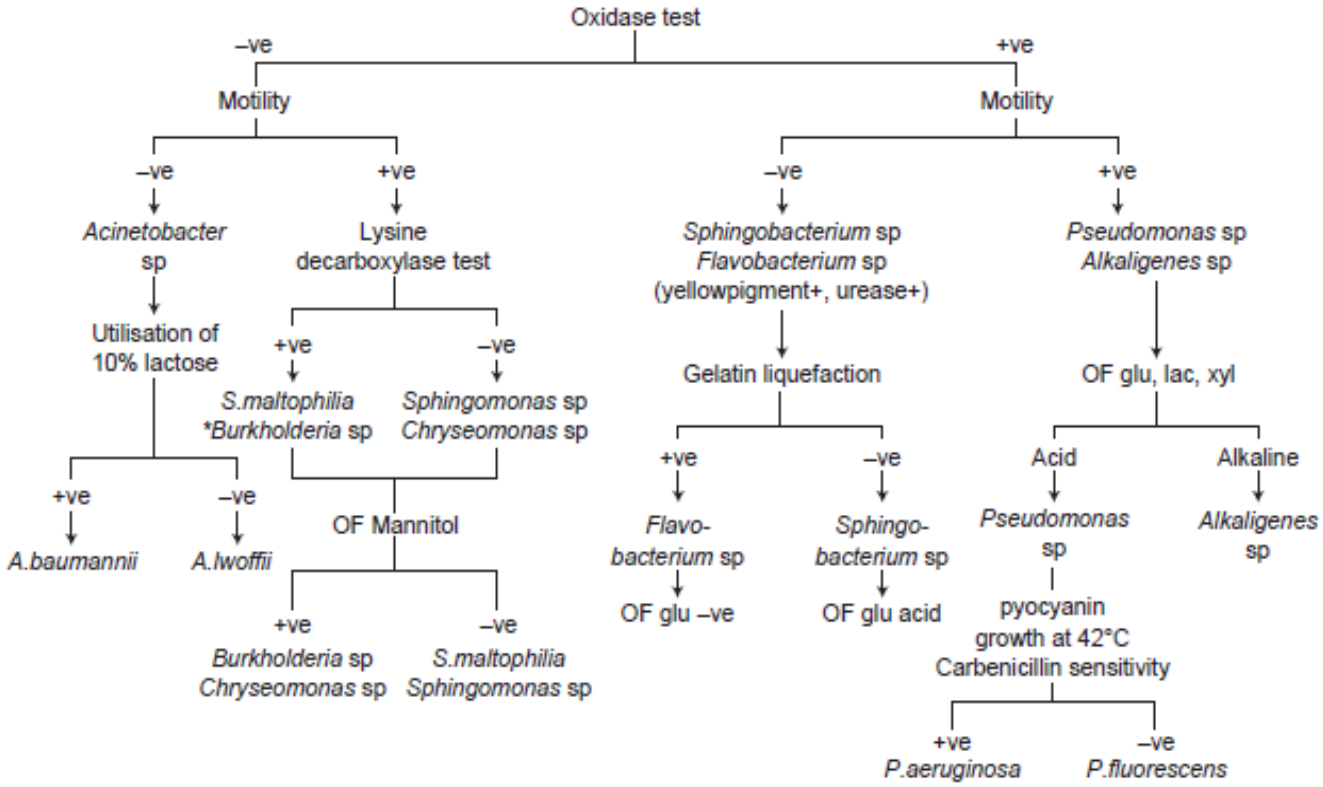


Figure 1: Scheme used in the study for identification of nonfermenting gram-negative bacilli * *Burkholderia* species vary in their oxidase reaction: *Burkholderia gladioli* is oxidase -ve, *B. cepacia* complex is weak +ve and *B. pseudomallei* is oxidase +ve, OF: Oxidation-fermentation[2].

4.5.4. IDENTIFICATION OF ISOLATES FROM ENVIRONMENTAL SAMPLES:

Pseudomonas aeruginosa colonies will appear green to blue-green, in Pseudomonas isolation agar with pigment that diffuses into the medium [97].

Acinetobacter species colonies will appear as pink mucoid colonies in Leeds Acinetobacter medium with pink color diffused into the medium [98].

4.6. ANTIBIOTIC SUSCEPTIBILITY TESTING

Antimicrobial susceptibility testing was performed by using Kirby Bauer disc diffusion technique according to CLSI 2014 guidelines. The following antibiotics were used for susceptibility testing: Ceftazidime, Cefepime, Ciprofloxacin, Levofloxacin, Gentamicin, Amikacin, Piperacillin-Tazobactum, Meropenem, Imipenem,Aztreonam. The *P.aeruginosa* ATCC 27853 strain was used as the quality control. All the discs were procured commercially (Hi-media laboratories limited).The diameter of the zone of inhibition was measured and interpreted according to the CLSI 2014 guidelines [91].

Imipenem resistant isolates (≤15 for *P.aeruginosa* and ≤18 for *Acinetobacter baumannii*) were further subjected to the following tests for Metallo beta lactamase detection.

4.7 PHENOTYPIC METHODS FOR DETECTION OF MBL:

4.7.1. IMIPENEM-EDTA COMBINED DISC TEST:

One imipenem (10µg) disc and one imipenem-EDTA disc were placed on the surface of an agar plate. Plates were incubated for 16 to 18 hours at 35˚C. If zone of inhibition of imipenem- EDTA disc was ≥ 7 mm more than that of imipenem disc alone, it was considered MBL positive.

4.7.2. IMIPENEM EDTA DISK SYNERGY (EDS) TEST:

EDTA disk synergy (EDS) test was done with simultaneous testing of two different β-lactams (imipenem and ceftazidime), for detection of metallo-β-lactamases in the imipenem resistant isolates.

A 0.5 M EDTA solution was prepared by dissolving 186.1 g of disodium EDTA $2H_2O$ in 1,000 ml of distilled water. The pH was adjusted to 8.0 by using NaOH and was sterilized by autoclaving. An overnight liquid culture of the test isolate was adjusted to a turbidity of 0.5 McFarland standard and spread on the surface of a MHA plate. A 10 μg imipenem disk or 30 μg ceftazidime disc was placed on the agar. A blank disk (6 mm in diameter, Whatmann filter paper no. 1 was kept on the inner surface of the lid of the MHA plate and 10 μl of 0.5 M EDTA was added to it. This EDTA disk was then transferred to the surface of the agar and was kept 10 mm edge-to edge apart from the imipenem or ceftazidime disk. After incubating overnight at 37^oC, the presence of an expanded growth inhibition zone between the two disks was interpreted as positive for MBL production

4.7.3.MBL E-TEST:

A 0.5 McFarland's suspension of the each isolate was made separately and inoculated on a plate of MHA. The Etest MBL strip containing an antibiotic concentration gradient for imipenem (IP) alone of 4 to 256 μg/mL and imipenem in combination with a fixed concentration of EDTA (IPI) gradient of 1 to 64 μg/mL is applied on the plate. The plate was then incubated at 37 °C for 24 h according to the manufacturer's instructions (Etest MBL). MIC ratio of IP (Imipenem)/IPI (Imipenem-EDTA) of > 8 or > 3 log 2 dilutions, a phantom zone or a synergistic zone indicated MBL production [99]

4.8. MBL GENE IDENTIFICATION:

bla_{VIM} and bla_{IMP} gene detection was done for ten isolates imipenem resistant *P.aeruginosa* and *A.baumannii* using multiplex PCR in Chromopark Research centre, 62-B, near NKR college, Trichy main road, Namakkal-637001, Tamil Nadu.

Procedure:

Multiplex PCR amplification for the simultaneous detection of blaIMP and blaVIM metallo beta -lactamase genes was carried out using Taq DNA polymerase, deoxynucleoside triphosphate and gene-specific primers (shrimpex, India).

The composition of the reaction mixture was as follows: Each PCR reaction mixture (25µl) contained 2µl of template DNA (plasmid DNA), 10 µl of 10 X PCR buffer, 0.5 µl of (0.5 µM) each of the primers and 9.0 µl of molecular grade water.

The PCR program was performed in a Thermal Cycler and it consisted of an initial incubation of 10 min at 37°C and an initial denaturation step at 94°C for 5 min, followed by 30 cycles of DNA denaturation at 94°C for 1 min, primer annealing at 54°C for 1 min, and primer extension at 72°C for 1.5 min. Following PCR, aliquots (20 µl) of the reaction mixtures were analyzed by electrophoresis on a 1.5% Agarose gel, containing ethidium bromide (0.2 mg/ml), in the presence of an appropriate DNA molecular weight marker. Then observe the amplification bands under UV Transilluminater and detection of resistance genes with the use of marker.

RESULTS

5.0 RESULTS

The study entitled "IDENTIFICATION OF NON FERMENTING GRAM NEGATIVE BACILLI FROM CLINICAL, ENVIRONMENTAL SAMPLES, THEIR ANTIMICROBIAL RESISTANCE AND DETECTION OF blaVIM/blaIMP GENES IN IMIPENEM RESISTANT ISOLATES was carried out in the department of microbiology , Chennai medical College Hospital and Research centre.

A total of 172 NFGNB isolates were identified from clinical samples processed during a period of 10 months from August 2014 to May 2015

5.1.Age wise distribution:

The agewise distribution of NFGNB is shown in Table

TABLE- 5: AGE WISE DISRIBUTION

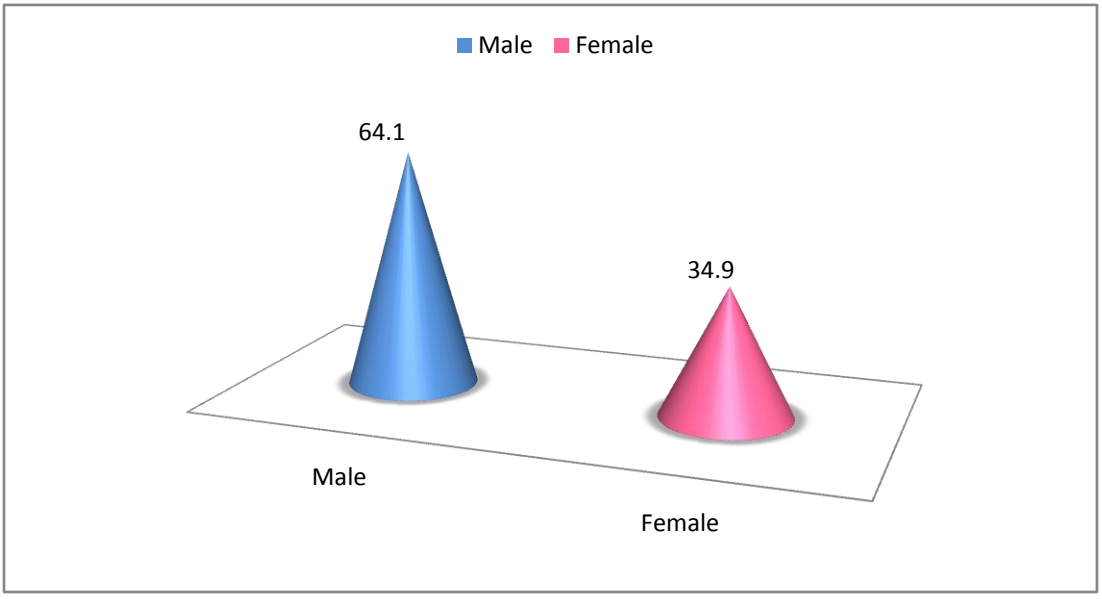
AGE IN YEARS	NO.OF SAMPLES	PERCENTAGE
1 – 10	7	4.1
11 – 20	12	7.0
21-30	19	11.0
31-40	32	18.6
41-50	30	17.4
51-60	28	16.3
>60	44	25.6
Total	172	100.0

The youngest patient was 6yr old and the oldest patient was 82yrs old. The age group above 60 years accounted for 25.6% which is the highest percentage followed by 31-40(18.6%),41-50(17.4%) and 51-60(16.3%) and the least percentage was seen below 10 years of age.

5.2. SEX WISE DISTRIBUTION

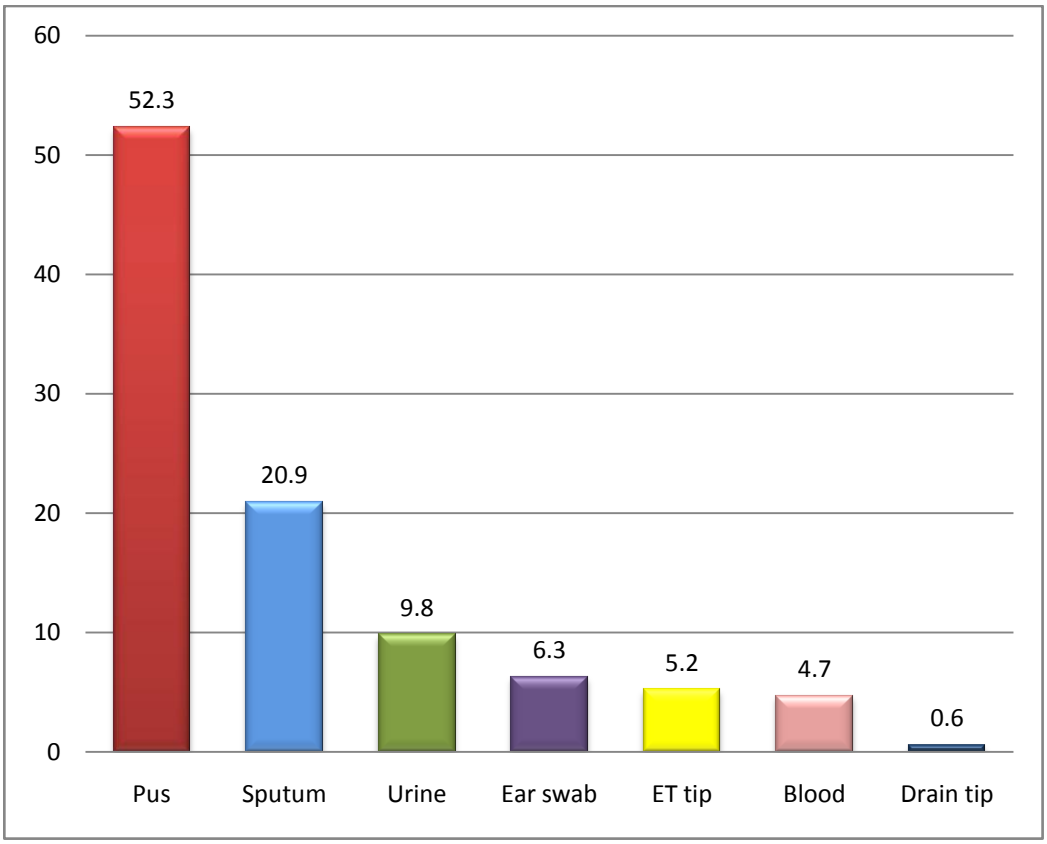
Out of 172 samples isolated 112(64.1%) were from males, 60(34.9%) were from females. Figure 1 depicts sex wise distribution of NFGNB. Sample wise distribution of isolates is depicted in figure 2.

Figure 1: Sex wise distribution of NFGNB



5.3. SAMPLE WISE DISTRIBUTION:

Figure 2: Sample wise distribution of NFGNB

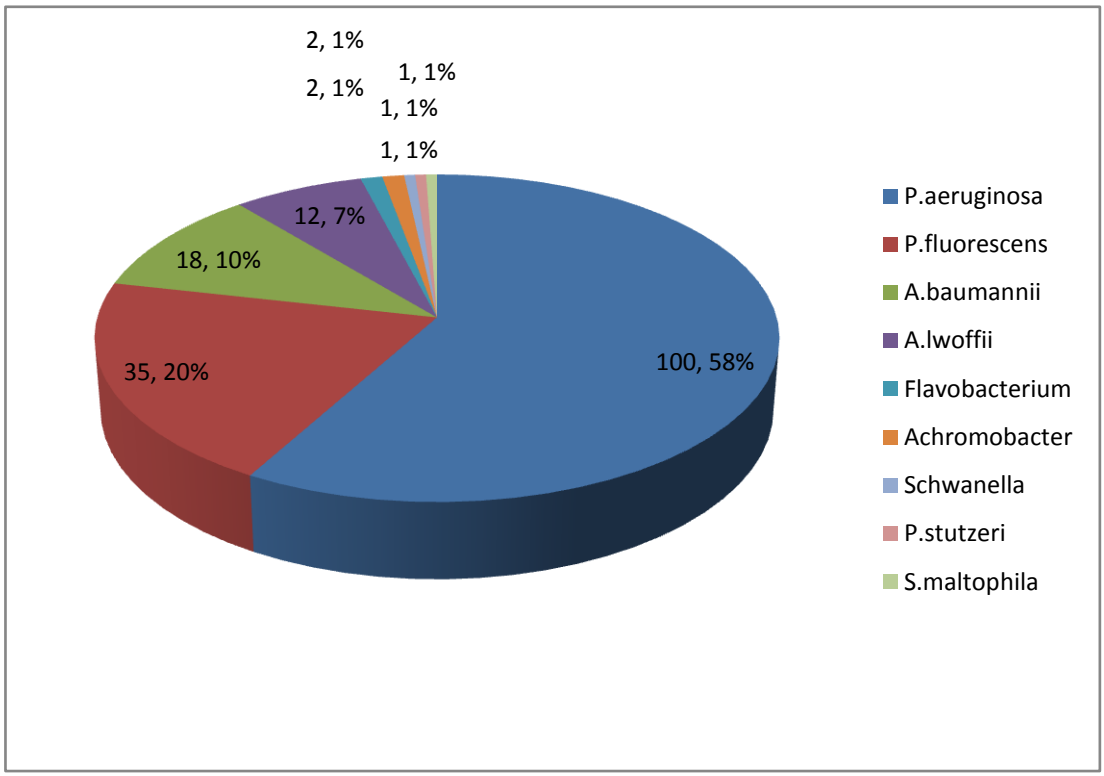


The above figure shows the sample wise distribution of NFGNB. Pus was the commonest sample from which NFGNB were isolated the most 52.3% followed by sputum (20.9%), urine (9.9%), ear swab (6.4%), ET tip (5.2%), blood (4.7%) and drain tip (0.6%).

Out of 172 isolates, the commonest was *P.aeruginosa* (100:59%) followed by *A.baumannii* (35:20.3%), *A.lwoffii* (18:10%), *P.fluorescens* (12:7%), *Flavobacterium*

meningosepticum and *Achromobacter* species (2 no.) and one isolate each of Shewanella and *P.stutzeri*, *Stenotrophomonas maltophila.*

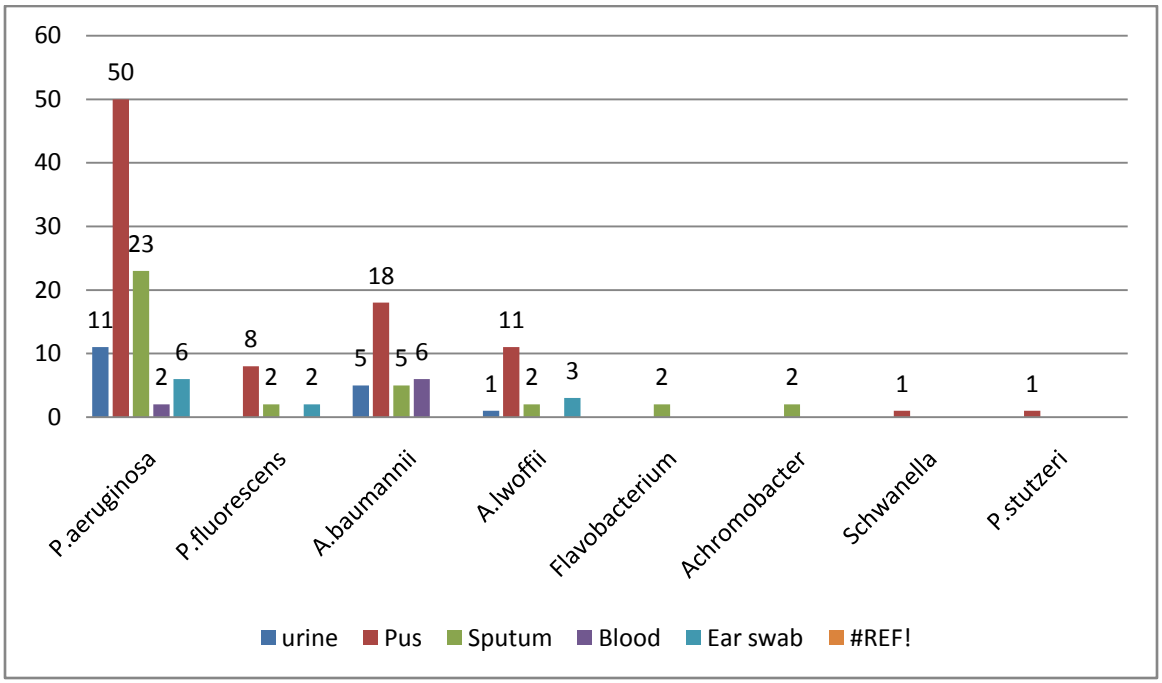
Figure 3: Distribution of NFGNB (Number & Percentage)



.

5.5. SAMPLE WISE ANALYSIS OF ISOALTES:

Figure 4: Sample wise analysis of Isolates



The above chart depicts the number of different isolates from various clinical samples. *P.aeruginosa* was mostly isolated from pus sample (50%), followed by sputum (23%), urine (11%), ET tube(11%), ear swab(6%), and blood.

P.fluorescens was mostly isolated from Pus(66%), followed by Sputum(16%) and ear swab(16%)

A.baumannii was isolated most commonly from pus(51.4%), followed by blood(15.7%) and urine(14.2%) and sputum (14.2%)

Most of the *A.lwoffii* were isolated from pus(61%),ear swa(16.6%), sputum, urine and drain tip.

Flavobacterium sp. and *Achromobacter* were identified only in sputum and *Shewanella* sp. and *P.stutzeri* were isolated only in pus samples.

5.6. SAMPLE WISE SENSITIVITY PATTERN OF P.AERUGINOSA ISOLATES:

Table 6: Sample wise sensitivity pattern of *P.aeruginosa* isolates

Antibiotics	PUS n=50		SPUTUM n=23		URINE n=11		BLOOD n=2		EAR SWAB n=6		ET Tube n=7	
	S (%)	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (in no.)**	**R (in no.)**	**S (in no.)**	**R (in no.)**	**S (in no.)**	**R (in no.)**
Ciprofloxacin	**60**	**40**	**91**	**9**	**81**	**19**	**2**	**0**	**5**	**1**	**7**	**1**
Levofloxacin	**60**	**40**	**86**	**14**	**81**	**19**	**1**	**1**	**5**	**1**	**7**	**1**
Amikacin	**64**	**36**	**100**	**0**	**81**	**19**	**1**	**1**	**5**	**1**	**6**	**2**
Gentamicin	**56**	**44**	**91**	**9**	**72**	**28**	**1**	**1**	**6**	**0**	**4**	**4**
Ceftazidime	**60**	**40**	**86**	**14**	**72**	**28**	**1**	**1**	**6**	**0**	**7**	**1**
Cefepime	**64**	**36**	**91**	**9**	**81**	**19**	**2**	**0**	**6**	**0**	**6**	**2**
Pipericillin Tazobactum	**76**	**24**	**95**	**5**	**81**	**19**	**2**	**0**	**6**	**0**	**7**	**1**
Aztreonam	**74**	**26**	**95**	**5**	**81**	**19**	**1**	**1**	**6**	**0**	**8**	**0**
Imipenem	**78**	**22**	**100**	**0**	**72**	**28**	**2**	**0**	**6**	**0**	**7**	**1**
Meropenem	**78**	**22**	**95**	**5**	**72**	**28**	**2**	**0**	**6**	**0**	**7**	**1**

P.aeruginosa showed the highest sensitivity to imipenem(78%) and meropenem(78%) in pus samples, to Amikacin(100%) in sputum samples, to Fluoroquinolones, cefepime, pipericillin-Tazobactum, Aztreonam in urine samples.

5.7. SAMPLE WISE SENSITIVITY PATTERN OF A.BAUMANNII ISOLATES:

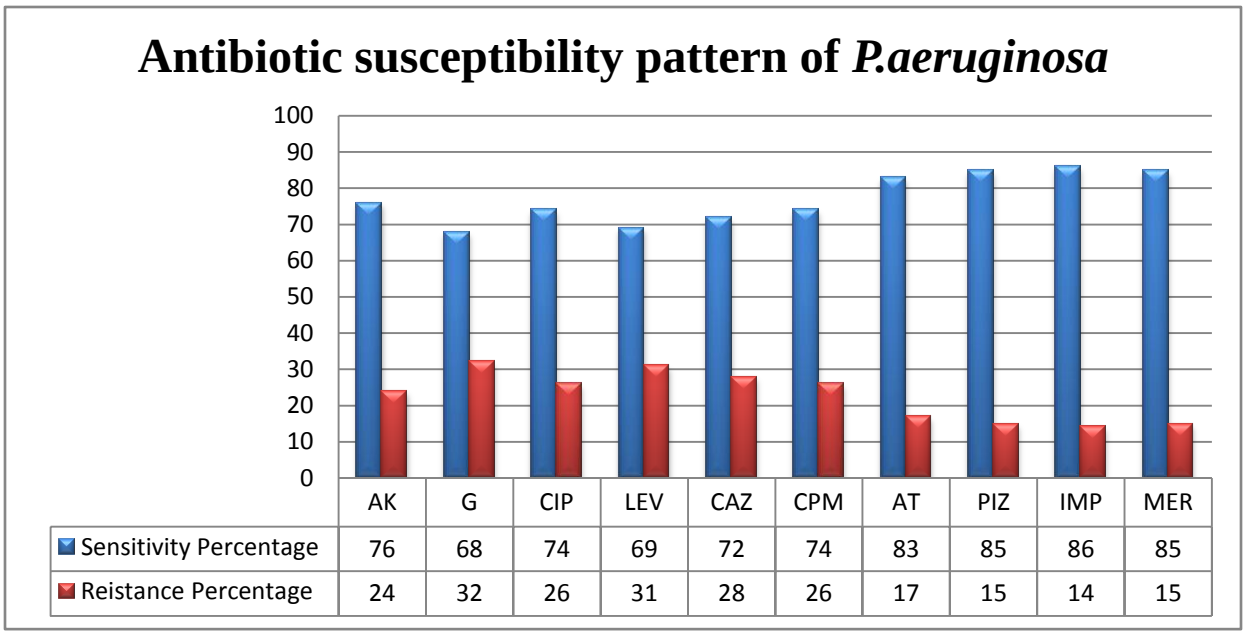
Table 7: Sample wise sensitivity pattern of *A.baumannii* isolates

Antibiotics	PUS n=18		SPUTUM n=5		URINE n=5		BLOOD n=6		ET Tube n=1	
	S (%)	R (%)	S (in no.)	R (in no.)	S (in no.)	R (in no.)	S (in no.)	R (in no.)	S (in no.)	R (in no.)
Ciprofloxacin	44	56	4	1	3	2	4	2	1	0
Levofloxacin	44	56	3	2	3	2	5	1	0	1
Amikacin	38	62	4	1	4	1	5	1	0	1
Gentamicin	38	62	3	2	3	2	5	1	0	1
Ceftazidime	16	84	3	2	3	2	4	2	0	1
Cefepime	22	77	2	3	2	3	5	1	0	1
Pipericillin Tazobactum	22	77	3	2	3	2	6	0	0	1
Aztreonam	77	22	5	0	5	0	5	1	1	0
Imipenem	33	67	2	3	2	3	6	0	0	1
Meropenem	33	67	2	3	2	3	6	0	0	1

The above table depicts the number of *A.baumanii* isolated from each sample and their susceptibility and resistance rates in relation to each sample.

5.9. ANTIBIOGRAM OF P.AERUGINOSA ISOALTES:

Figure 5: Antibiotic susceptibility pattern of *P.aeroginosa* isolates from all Clinical samples

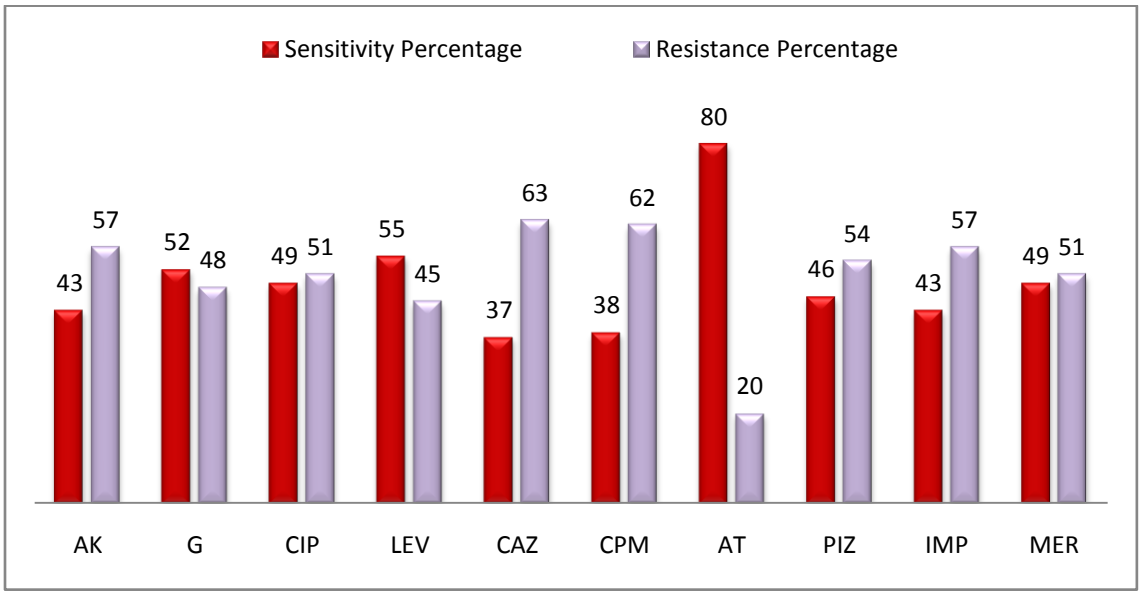


	AK	G	CIP	LEV	CAZ	CPM	AT	PIZ	IMP	MER
Sensitivity Percentage	76	68	74	69	72	74	83	85	86	85
Reistance Percentage	24	32	26	31	28	26	17	15	14	15

Eighty six percentage of isolates were sensitive to imipenem, followed by piperacillin tazobactum and meropenem (85%). 74% of isolates were sensitive to ciprofloxacin and cefepime and 76% sensitivity to amikacin. A sensitivity of 69% and 68% were observed for levofloxacin and gentamycin respectively. The most effective drug in our study was imipenem.

5.10. ANTIBIOGRAM OF A.BAUMANNII ISOLATES:

Figure 6: Antibiotic susceptibility pattern of A.baumanii isolates from all clinical samples



Eighty percent of the isolates were sensitive to Aztreonam. Sensitivity of the drugs gentamycin, levofloxacin, ciprofloxacin and meropenem were 52%, 55%, 49% and 49% respectively. Resistant was observed more for the drugs ceftazidime (63%), cefipime (62%), amikacin (57%) and imipenem (57%), ciprofloxacin (51%) and meropenem (51%).

Among 100 *P.aeruginosa* isolates, 14 (14%) were resistant to imipenem, and 15 isolates (15%) were resistant to meropenem. Among 35 *A.baumannii* isolates, 20 (57%) were resistant to imipenem and 18 (51%) were resistant to meropenem. These isolates were further tested by phenotypic methods for detection of MBL production like

combined disc test (imipenem/imipenem+EDTA), double disc synergy test ans MBL E test (IP-IPE)

5.11. PHENOTYPIC METHODS FOR MBL DETECTION

The different phenotypic methods for MBL detection is shown in Table 8.

Table 8: Phenotypic methods for MBL detection

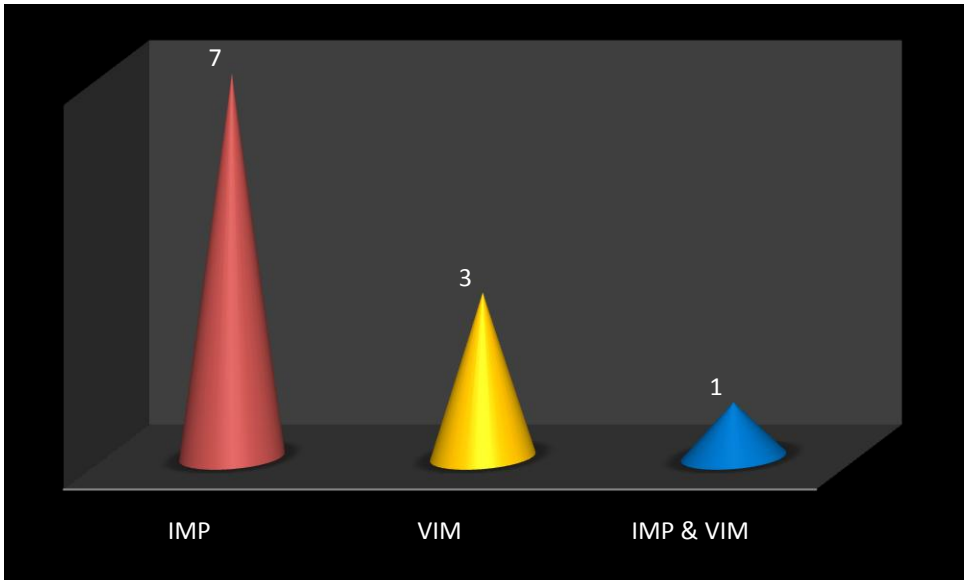
Test	no. of isolates tested	Isolates positive
Combined disc test	10	6
Disc synergy test	10	8
MIC E-strip	10	10

Three phenotypic tests were performed for the detection of Metallo bta lactamase among ten imipenem resistant isolates. (3-P.aeruginosa and 7-A.baumanii) In that, 6 isolates were positive for MBL in combined disc test, and 8 showed positivity for MBL in double disc synergy test and all the 10 isolate were MBL positive in MBL E-test.

5.12 Genotype of MBL producing isolates:

Genotyping for the detection of $bla_{IMP,}$ bla_{VIM} genes was performed for the ten isolates which showed presence of MBL in the above phenotypic tests using multiplex PCR. The results of the test are depicted in the following figure 7.

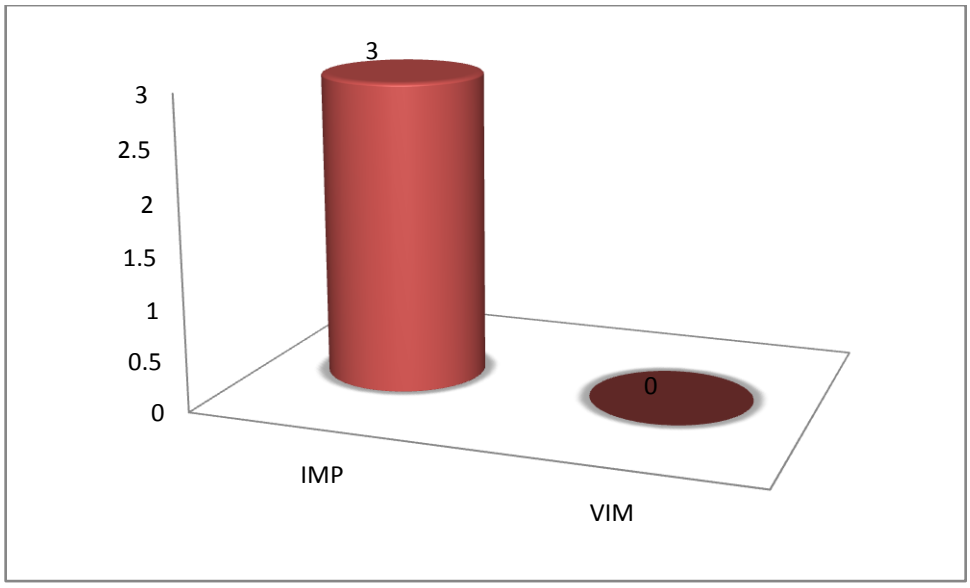
Figure 7: Number of $bla_{IMP,}$ bla_{VIM} genes among Imipenem resistant isolates



Among ten phenotypically confirmed isolates, bla_{IMP} gene was detected in 7 isolates and bla_{VIM} gene was detected in 3 isolates and both the genes were present in 1 isolate.

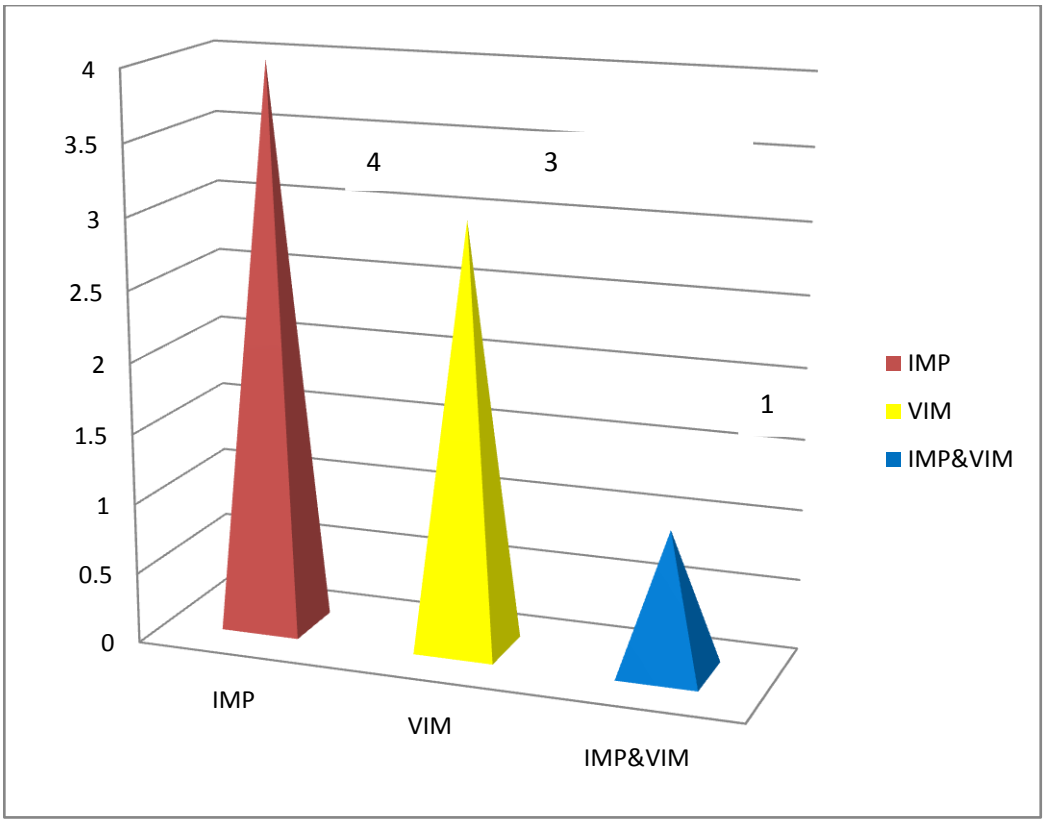
Among three imipenem resistant *P.aeruginosa* isolates, all the three isolates possessed bla_{IMP} gene. None of the isolates possessed bla_{VIM} gene. Figure 8 depicts number of $bla_{IMP,}$ bla_{VIM} genes among Imipenem resistant *P.aeroginosa* isolates.

Figure 8: Number of $bla_{IMP,}$ bla_{VIM} genes among Imipenem resistant *P.aeroginosa* isolates



Among seven imipenem resistant A.baumannii isolates, four possesed bla_{IMP} gene and , three isolates possesed bla_{VIM} gene and one possesed both bla_{VIM} gene and bla_{IMP} gene. Figure 9 depicts number of $bla_{IMP,}$ bla_{VIM} genes among Imipenem resistant *A.baumanii* isolates.

Figure 9: Number of $bla_{IMP,}$ bla_{VIM} genes among Imipenem resistant *A.baumanii* isolates



5.13. ENVIRONMENTAL SAMPLES:

Among sixty environmental samples, P.aeruinosa was isolated in 5 sites including SICU tap water, sink, Post operative ward door handle, sink, burns ward door handle and Acinetobater baumannii was isolates from two sites including ICU bed rails and RICU sink.

All strains isolated were sensitive strains.

IMAGES

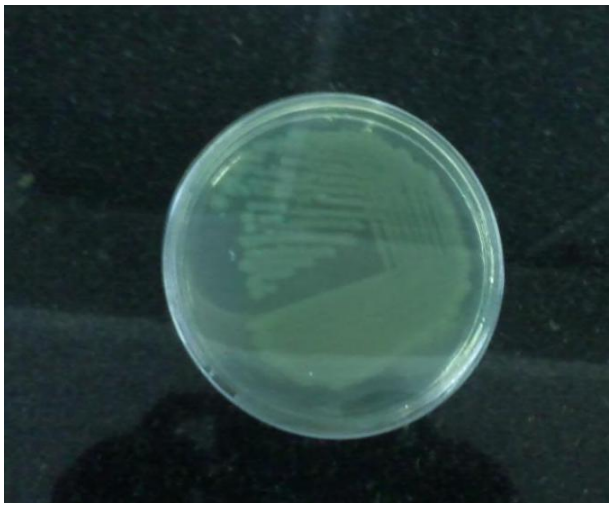


Fig 10: Pyocyanin pigment of *P.aeruginosa* in Pseudomonas isolation agar

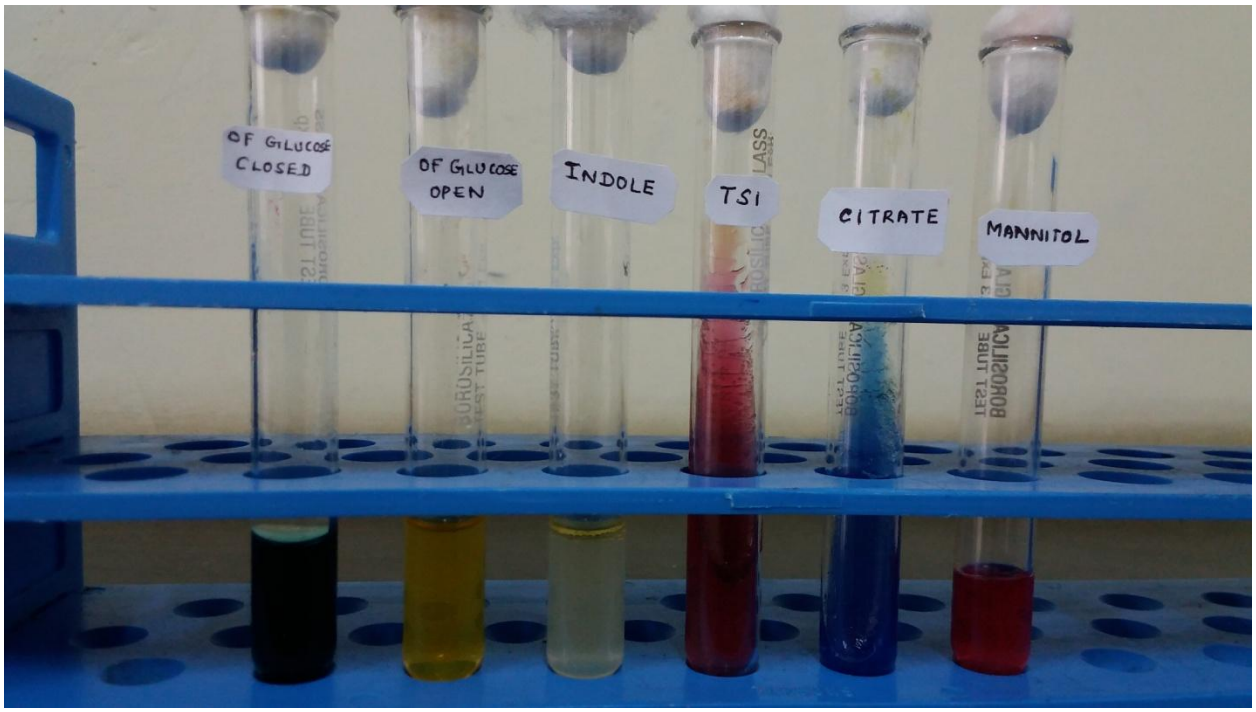


Fig 11: Biochemical reactions of *P.aeruginosa*

OF glucose showing fermentative reaction

Indole – Negative; TSI – Alkaline slant/No change;
Citrate Utilised; Mannitol- Not fermented

Fig 11: Acinetobacter baumanii on Mac conkey plate showing pinkish tint

Fig 12: Biochemical reactions of A.baumanii

Arginine dihydrolase – Positive ; 10% lactose - fermented

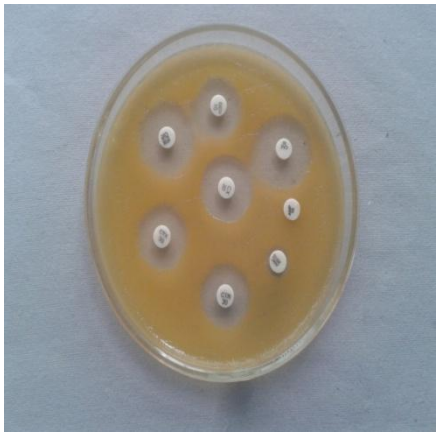


Fig 13: Yellow pigment of Flavobacterium species in Muller Hinton plate

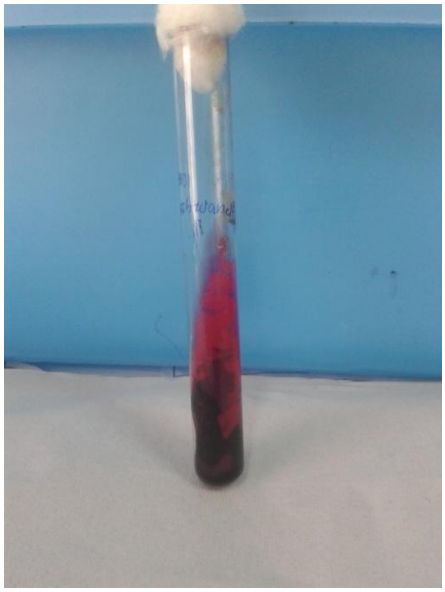


Fig 14: Formation of Hydrogen sulfide in TSI agar by H_2S producing Shewanella species

Fig 15: EDTA DOUBLE DISC SYNERGY TEST

Enhancement of zone towards EDTA disc

E – EDTA disc

P – Plain disc

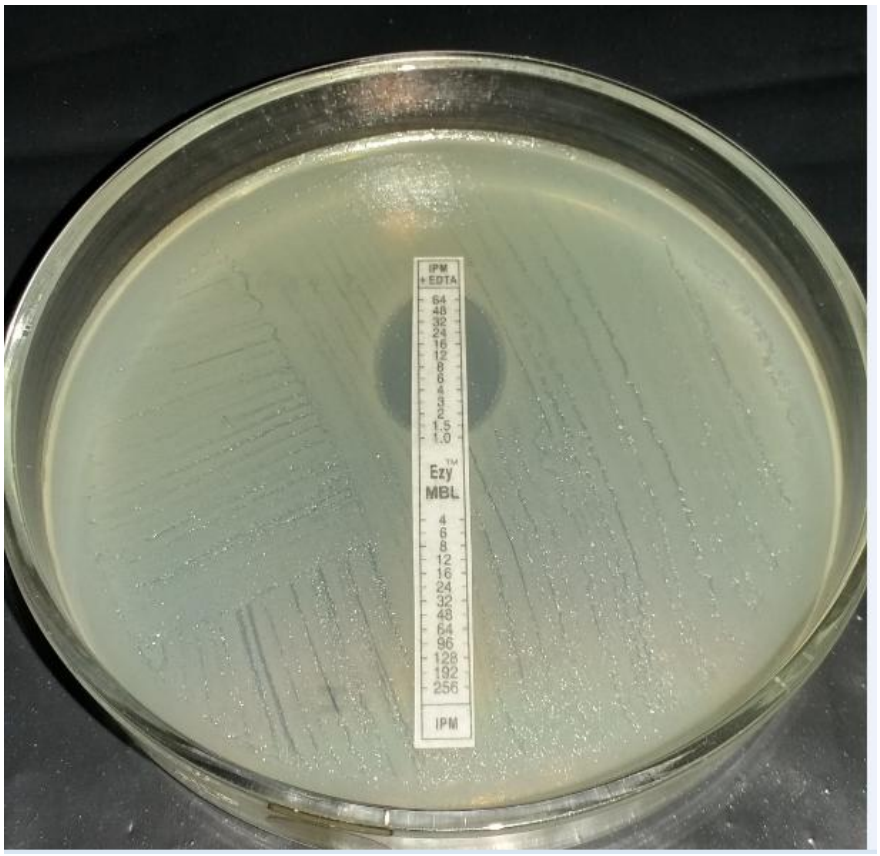
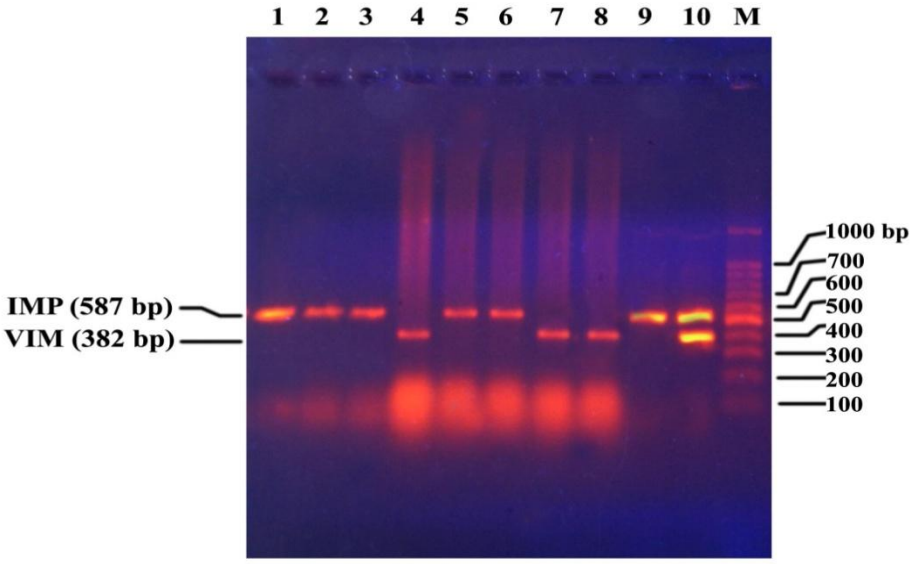


Fig 16: MBL POSITIVE TEST ISOLATE

$$\frac{\text{IPM}}{\text{IPM+EDTA}} = > 8, \text{ MBL POSITIVE}$$

Amplification of IMP and VIM gene by multiplex PCR



.

DISCUSSION

6.0. DISCUSSION

NFGNB are known to cause hospital acquired infections and opportunistic infections. The multidrug resistant organisms have increased due to widespread and indiscriminate use of antibiotics. This results in increased economic burden, duration of hospital stay and increased mortality and morbidity.

In this study, NFGNB were identified and their mechanisms of resistance to imipenem and meropenem drugs were detected.

6.1. AGE WISE DISTRIBUTION:

In our study the highest percentage was seen in the age group above 60 years which is 25.6% which is similar to other studies conducted by Kalidas Rit et al [4] in Kolkata in 2013 where 72% of the patients were adults over 45 years of age and a study by Mohammed Rahbar [5] where majority of the patients (70%) were adults and study by Meenakumari et al [100] where 90% were adults. This could be due to sub normal immune system.

But this is in discordance with a study by Chander Anil et al in 2013 [101] and a study by Rashid et al [102] where the highest percentage is seen in the age group between 21 to 40 years.

6.2. RISK FACTORS:

The various risk factors observed in our study in decreasing order of frequency are

1. Urinary catheterization -35%
2. Diabetes Mellitus – 12%
3. Stay in ICU – 12%
4. Surgery – 11%
5. Burns – 6%
6. Mechanical ventilation – 5%
7. Prolonged hospital stay - 5.2%
8. Trauma – 2%

The percentage of risk factor varies in a study by Mohammed Rahbar [5] where 69% had history of invasive procedures, 56.9% had previous history of hospitalization, 39% were under mechanical ventilation, 38% of patients were hospitalized in ICU.

Another study by Mohanasundaram [103] in 2010 revealed the following risk factors.

24% had previous surgery, (19.4%) Road traffic accident, 13.5% had underlying renal disease and diabetes mellitus, 11% with underlying respiratory disease and (6%) burns.

A study by Kalidas Rit et al, [4] showed that the various risk factors involved are connection to ventilator, history of invasive procedures, steroid usage, under immunosuppressive therapy. The percentage was not mentioned.

6.3. SEX WISE DISTRIBUTION:

In our study males (64.1%) are more commonly affected than the females (34.9%). Similar observation was made in other studies conducted by Murugan 2010 [104] where the male to female ratio was 3:1 and in a study by Kalidas et al [4] 55% were males and 45% were females and in a study in 2012 by Piyush [105] 71.5% were males and 28.5% were females.

6.4. SAMPLE WISE DISTRIBUTION:

In the present study, NFGNBS were most commonly isolated from pus samples (52.3%) followed by sputum (20.9%), urine (9.9%), ear swab (6.4%), ET tip (5.2%), blood (4.7%) and drain tip (0.5%). This is in concordance with a study conducted in 2009 in Kolar, India wherein NFGNBs were most commonly isolated from pus samples. Out of 193 samples, 120(62.2%) isolates of NFGNB were from pus samples [2]. Another study conducted in Belgaum in 2007 also showed that maximum number of NFGNB were from pus samples (58.4%) [106].

But it is discordant with the study carried out by Meenakumari et al [100] in 2011 which showed most of the NFGNBs were from respiratory samples(52.9%)and only 9.8% were from pus samples and a study by Mohammad Rahbar et al [5] in 2010 where 34% was from tracheal aspirates. This may be due to nature of selection of samples.

6.5. ISOLATE WISE DISTRIBUTION:

In our study *P.aeruginosa* (59%) was the most common organism isolated from the clinical samples, followed by *A.baumannii* (21%), *A.lwoffii* (18:10%), *P.fluorescens* (12:7%), *Flavobacterium meningosepticum* and *Achromobacter* species (2 no.) and one isolate each of Shewanella, *P.stutzeri*, and *Stenotrophomonas maltophila.* This result is in concordance with a study conducted by Kaidas Rit et al [4], 2013 where the predominant isolate was *P.aeruginosa* (50.2%) followed by *A.baumanii* (24.8%), *Acinetobacter lowffii* (5.47%), *Pseudomonas fluorescens* (1.49%), *Pseudomonas stutzeri* (1.99%),.

Other studies showing similar results include study by Chawla K et al [3] in 2013, where 56.7% isolates were *P.aeruginosa* and 39.3% were *Acinetobacter* sp and a study by Gokale in 2012 [106] where *P.aeruginosa* constituted 82.3% and *A.baumannii* 15.4%.

6.6. SAMPLE WISE DISTRIBUTION OF ISOLATES AND THEIR ANTIBIOGRAM:

NFGNB shows a wide spectrum of antibiotic susceptibility.

6.6.1. Pus sample :

In this study, *P.aeruginosa*(55.5%) and *A.baumannii*(20%) were the most common isolates from pus samples. This is similar to the study by Rashid et al,.[102] where 55.1% of *P.aeruginosa* isolates were from pus samples and a study by Malini et al [4] , where *P.aeruginosa* and *A.baumannii* were the predominant isolates from pus samples.

In our study, *P.aeruginosa* showed the highest sensitivity to imipenem(78%) and meropenem(78%) followed by Pipericillin Tazobactum(76%) and Aztreonam (74%) and

more resistance was observed to Gentamicin(44%) followed by ceftazidime (40%), Ciprofloxacin(40%) and levofloxacin(40%)

Study by Rashid et al [102] showed *P.aeruginosa* from pus samples showed 100% resistant to azithromycin (100%), ceftazidme(86%),ciprofloxacin(75%) and least resistant to imipenem (3%)

A.baumanii showed more sensitivity to Aztreonam(77%) and more resistant to ceftazidime followed by imipenem(77%) and meropenem(67%)

6.6.2.Urine samples :

In our study, the predominant isolate was *P.aeruginosa*(11%) followed by *A.baumannii*(14.2%). This is similar to the study conducted in Manipal in 2011 by Shobha [6] where the commonest isolate was *P.aeruginosa* (7.6%), followed by *Acinetobacter* sp.(1.8%).

In our study *P.aeruginosa* 81% isolates were sensitive to Amikacin, Aztreonam, Ciprofloxacin, Levofloxacin, Pipericillin-Tazobactam, and 72% isolates sensitive to imipenen. The sensitivity pattern varies in a study at Manipal [2] where the isolate is only 42% sensitive to Gentamicin, 36% sensitive to Ceftazidime and 100% resistant to Aztreonam.

In our study, *A.baumannii* showed 100% sensitivity to Aztreonam which is in discordant with the study at Manipal [2] where none of the isolates were sensitive to Aztreonam.

6.6.3.Respiratory samples:

Out of 45 samples from respiratory tract, 36 sputum and 9 endotracheal tube tips, the predominant isolate in our study is *P.aeruginosa* (68%) followed by *A.baumannii* (13%).

This is in discordant with a study by Malini et al,[2] where the predominant respiratory pathogen is *A.baumannii* (40%)

In our study the most active drug against *P.aeruginosa* from sputum sample was Amikacin (100%) followed by Pipericillin-Tazobactam (95%). Study by Piyush [105] in Allahabad showed the most active antibiotic is Pipericillin-Tazobactam (91%) followed by Imipenam (89%)

But a study by Rashid et al [102] showed that 100% of *P.aeruginosa* from tracheal aspirates were resistant to amikacin, ciprofloxacin, and ceftazidime.

6.6.4.Ear swab:

In our study the most common isolate from ear swab is *P.aeruginosa* (12%). The rate of resistance to ciprofloxacin, amikacin is 16.6%. This result is similar to the study by Rashid et al [102] were 10.5% of *P.aeruginosa* were isolated from aural swabs. The rate of resistance to ciprofloxacin and amikacin is 13.3% and 9.7% respectively.

6.7. Overall Antibiotic susceptibility of *P.aeruginosa*:

In our study, among *Pseudomoans aeruginosa* species, imipenem, meropenem, pipercillin-tazobactum, Aztreonam, cefepime showed highest activity with overall susceptibility of 86%, 85%, 85%, 83%, 74% respectively. The sensitivity to imipenem is similar to study conducted by Mohammad Rahbar 2010 [5] where it was 78.9%, and 94.2% in a study by Malini et al in the year 2009 [2].

This result was in discordance with a study conducted in 2013 by Juyal,[7] where the highest sensitivity was observed with Amikacin (72.3%).

In our study the highest resistance was recorded for gentamicin (32%), levofloxacin (31%). But in a study by Tripathi et al [105] in 2010, ceftazidime showed highest resistance (36%).

6.8. Overall Antibiotic susceptibility of *Acinetobacter baumannii:*

In our study, *A.baumannii* showed resistance to most of the antibiotics used. The most active antibiotic against *A.baumanii* was Aztreonam and 80% of isolates were susceptibile to this antibiotic. The highest resistance was seen to drugs like Amikacin (57%), Imipenem (57%) and Pipericillin Tazobactum(54%) . This is in discordance to other studies by Mohammad Rahbar [5] in 2010 where imipenem(95.5%) was the most active drug and 90% of the isolates were resistant to Aztreonam. In another study by Kalidas Rit et al in 2013[4] where the most effective drug was Imipenem with susceptibility of 95%.

6.9. MBL Detection:

Active surveillance of multi drug resistant organism is very important for provision of effective health care. MBLs are emerging as one of the most troublesome resistant mechanisms because they limit our treatment options and also the genes responsible for resistance is carried on highly mobile elements resulting in early dissemination.

In present study, MBL detection was performed for 10 imipenem (3-P.aeruginosa and 7-A.baumanii) resistant isolates, using three different phenotypic methods like combined disc test (imipenem/imipenem+EDTA), double disc synergy test and MBL E-test (IP-IPE). Genotypic detection of bla_{VIM} and bla_{IMP} gene was done using multiplex PCR.

6.9.1. Sensitivity of different Phenotypic tests:

In our study, screening for MBL showed that 6 isolate were positive for MBL in combined disc test, and 8 showed positivity for MBL in double disc synergy test and all the 10 isolate were MBL positive in MBL E-test. Genotypic detection of MBL genes using multiplex PCR confirmed the presence of MBL genes in all the ten isolates.

This variation between phenotypic and molecular characterization is previously documented in studies conducted by Saber Yousefi in Iran in 2010 [107], where 39 isolates were positive for MBL by double disc synergy test but PCR confirmed the presence of MBL gene only in 24 isolates.

Another study by Pena et al in Portugal in 2008 [108] showed that 40 isolates were MBL positive in combined disc method but PCR results showed only 26 isolates were positive for MBL gene. The reason for this inconsistency is unknown. The possible reasons could be the interference of other resistance mechanisms with MBL detection by phenotypic methods or presence of other unrecognised MBL resistance genes [109 110].

In our phenotypic study, MBL E-test was found to be the most sensitive. This is in accordance to other studies by Behera 2008 [111] and Yousefi 2010 [107].

Study by Behera 2008 [111] showed that both combined disc test and E test are equally sensitive for MBL detection and combined disc test is easy and economical. But in our study combined disc showed least sensitivity compared to other tests.

Genotypic detection of bla_{VIM} gene and bla_{IMP} gene were done for ten isolates (7 were *A. baumannii*, and 3 were *P.aeruginosa*)

In our study, among 3 *Pseudomonas aeruginosa* isolates, all were positive for bla_{IMP} gene. None of the isolates was positive for bla_{VIM} gene.

This is similar to the study by Fallah [112] in 2013 where 6 *P.aeruginosa* isolates were positive for bla (IMP) gene, while bla(VIM) gene was not detected in any isolate.

But a study by Aghamiri in 2014[113] indicated that 20 (9%) of *P.aeruginosa* strains contained the IMP gene, whereas 70 (33%) of them harbored the VIM gene and a study by Hirataka 2003 [13] showed that bla_{IMP} positive *Pseudomonas aeruginosa* isolates were more frequently resistant to multiple drugs and cause more serious infections.

Among 7 *Acinetobacter baumannii* isolates, four were positive for bla_{IMP} gene and three for bla_{VIM} gene and one was positive for both bla_{VIM} gene and bla_{IMP} gene.

A study by Karthika 2009 [15] in Puducherry showed 42% of *Acinetobacter baumannii* isolates showed bla_{IMP} gene where none of the isolates showed bla_{VIM} gene.

The increase in the frequency of MBLs presents an emerging threat of complete resistance to the useful drugs against these isolates in India. Hence reserved and careful use of antibiotics, as well as strict hygiene practices, are critical for preventing the emergence of complete resistance and spread of this multi drug resistant organisms in this country

6.10. Environmental isolates:

Among sixty environmental samples, *P.aeruginosa* was isolated from 5 sites including SICU tap water, sink, Post operative ward door handle, sink, burns ward door handle and *Acinetobater baumannii* was isolated from two sites which included ICU bed rails and RICU sink.

But a study conducted by Fazeli H et al,.in 2012 [114] showed that 45 *P.aeruginosa* isolates were obtained from hospital means such as shower, tap water, patients room and hospital personnel.

Studies by Trautmann et al 2011, Bert et al 1998, Buttery et al 1998 [115] [116] [117] showed that *P.aeruginosa* was isolated from hospital tap water.

Studies by Pina et al 1998 [118] showed that *A.baumanii* was isolated from hospital environment.

In our study all the environmental isolates were sensitive strains. So there is no correlation between the hospital environmental samples and the drug resistant strains isolated from the clinical samples.

But a study conducted in Iran in 2012 [114] showed that there is similarity between the environmental and hospital strains of *P.aeruginosa* in phenotypic characters, antibiotic resistance pattern, PCR ribotyping pattern hence the hospital environment could be a main reservoir for *P. aeruginosa* infections.

SUMMARY

7.0. SUMMARY

This study entitled "IDENTIFICATION OF NON FERMENTING GRAM NEGATIVE BACILLI FROM CLNICAL, ENVIRONMENTAL SAMPLES AND THEIR ANTIMICROBIAL RESISTANCE AND DETECTION OF bla_{VIM}/bla_{IMP} GENE IN IMIPENEM RESISTANT ISOLATES" was carried out in the department of Microbiology, Chennai Medical College hospital and research centre, Trichy from August 2014 to May 2015.

- 172 isolates of NFGNB were obtained from the various clinical samples. 90 (52.3%) isolates were from pus, followed by 36 (20.9) from sputum, 17 (9.8%) from urine, 11 (6.3%) from ear swab, 9 (5.2%) from ET tip, 8 (4.7%) from blood, 1 (0.5%) from drain tip.
- *P.aeruginosa* and *A .baumanii* are the two common NFGNB isolated from the clinical specimens followed by *A.lwoffii* and *P.fluorescens*.
- The most effective drug in this study was found to be imipenem which showed sensitivity percentage of 79.1%
- The imipenem resistant isolates were detected for MBL resistance by using different phenotypic methods like combined disc test, Disc synergy test and MIC E-strip.
- Genotypic detecetion of bla_{VIM} gene and bla_{IMP} gene was done using multiplex PCR for 10 phenotypically confirmed isolates where bla_{IMP} was detected in 7 isolates (3-*P.aeruginosa*; 4-*A.baumannii*) and bla_{VIM} was

detected in 3 isolates (3-*A.baumannii*) and both the genes were detected in one isolate of *A.baumanii.*

- Among sixty environmental samples, *P.aeruginosa* was isolated in 5 sites including SICU tap water, sink, post operative ward door handle, sink, burns ward door handle and *Acinetobater baumannii* was isolates from two sites including ICU bed rails and RICU sink.
- All strains isolated were sensitive strains.

CONCLUSION

CONCLUSION

NFGNB are emerging as important opportunistic pathogens and are resistant to commonly used antibiotics.

The present study highlights the importance of speciating NFGNB and to know the resistance of carbapenems in these isolates.

Prevalance of pathogens often varies between hospitals, among different patients in the same hospital. So it is important for clinicians to remain updated with prevalance and antimicrobial susceptibility pattern of the circulating pathogens and the antibiotics to be used for empiric therapy should be selected accordingly.

More importantly these organisms have great potential to survive in hospital environment, so effective methods of sterilization and infection control measures like hand hygiene, using personal protective equipments, environmental cleaning and disinfection, sterilization and disinfection of various instruments and devices used in patient care and biomedical waste management should be implemented.

Continuous awareness of the need to maintain good housekeeping, equipment decontamination, strict attention to hand washing and isolation procedures and control of antibiotic usage especially in high risk areas should be implemented.

STRENGTH:

1. Genotyping was carried out apart from phenotypic methods.

2. Not only clinical samples but also environmental samples were processed.

LIMITS:

1. It is a single center study

2. Detection of MBL genes was done only for 10 imipinem resistant isolates.

3. Other antibiotic resistance mechanisms like ESBL detection, ampC detection were not performed

ANNEXURE I

MASTER CHART

S NO	ID NO	SEX	AGE	SAMPLE	ISOLATE	WARD	OP/IP	CIP	LEV	AK	G	CAZ	CPM	PIT	AT	IMI	MER	RES
1	1106246	F	26	ear swab	P.aeruginosa	ENT	OP	S	S	S	S	S	S	S	S	S	S	I
2	157438	F	12	Pus	P.aeruginosa	paediatric	IP	S	S	S	R	S	S	S	S	S	S	I
3	153410	F	65	Pus	A.baumannii	ortho	IP	R	S	S	S	R	R	R	R	R	S	I
4	157716	F	57	Pus	A.lwoffii	surgery	IP	S	S	R	S	S	S	S	S	S	S	I
5	1110008	M	63	Pus	P.aeruginosa	ENT	OP	R	S	S	R	R	R	S	S	S	S	I
6	156974	F	38	Pus	P.aeruginosa	ortho	IP	R	R	S	R	R	R	R	S	S	S	I
7	153694	M	59	Pus	P.aeruginosa	ortho	IP	R	R	S	S	R	S	S	S	S	S	I
8	158342	F	33	Pus	A.baumannii	burns	IP	R	R	S	R	R	R	R	S	S	S	I
9	154808	F	45	Pus	P.aeruginosa	OG	IP	S	R	S	S	S	S	S	S	S	S	I
10	159160	F	11	ear swab	P.fluorescens	ENT	IP	S	R	S	S	S	S	R	R	S	S	I
11	158193	M	78	Pus	P.aeruginosa	surgery	IP	S	S	S	S	S	S	S	S	S	S	I
12	158857	M	40	Pus	A.baumannii	thoracic medicine	IP	S	S	S	S	S	S	S	S	S	S	I
13	159308	M	34	ear swab	P.aeruginosa	ENT	IP	S	S	S	S	S	S	S	S	S	S	I
14	158141	M	57	Pus	P.aeruginosa	surgery	IP	R	S	R	R	S	S	S	R	S	S	I
15	158350	M	33	Pus	P.aeruginosa	burns	IP	S	S	S	S	S	S	R	R	S	S	I
16	157328	F	28	Pus	P.aeruginosa	burns	IP	S	S	R	R	S	S	S	S	S	S	I
17	1117931	M	32	ear swab	P.fluorescens	ENT	OP	S	S	S	S	S	S	S	S	S	S	I
18	160554	M	86	Pus	P.fluorescens	surgery	IP	S	S	S	S	S	S	R	S	S	S	I
19	160486	M	48	Pus	P.aeruginosa	surgery	IP	S	S	S	S	S	S	S	S	S	S	I

20	1133550	M	46	Pus	A.baumannii	surgery	OP	S	S	S	S	S	S	S	S	S	S	I
21	160540	M	43	Pus	A.baumannii	burns	IP	S	S	R	R	R	R	R	R	R	S	I
22	160404	M	25	Pus	P.aeruginosa	surgery	IP	R	S	R	R	R	R	S	R	S	S	I
23	160965	F	45	Pus	P.aeruginosa	thoracic medicine	IP	R	R	R	R	S	R	S	S	S	S	I
24	161108	M	22	Pus	P.fluorescens	ortho	IP	S	R	S	S	S	S	S	S	S	S	I
25	160549	M	38	Pus	P.aeruginosa	icu	IP	S	S	S	S	S	S	R	R	R	S	I
26	1612213	M	11	ear swab	A.lwoffii	paediatric	IP	R	R	S	S	S	S	R	S	S	S	I
27	161188	M	20	Pus	P.aeruginosa	ortho	IP	R	R	R	R	R	S	S	S	R	R	I
28	160133	M	61	Pus	P.aeruginosa	surgery	IP	S	R	S	S	S	S	S	S	S	S	I
29	182659	F	32	Pus	A.baumannii	surgery	IP	R	R	R	R	R	R	R	S	R	R	I
30	160333	M	34	Pus	P.aeruginosa	ortho	IP	S	S	S	S	R	R	R	S	R	R	I
31	162012	M	34	Pus	P.aeruginosa	surgery	IP	S	S	S	S	S	S	S	S	S	S	I
32	162829	M	65	Pus	A.baumannii	medicine	IP	R	S	R	R	R	R	R	S	R	R	I
33	163227	M	6	Pus	A.lwoffii	paediatric	IP	S	R	S	S	S	S	S	R	S	S	I
34	163337	M	10	Pus	P.aeruginosa	paediatric	IP	S	R	S	S	S	S	S	S	S	S	I
35	160133	M	61	Pus	P.aeruginosa	surgery	IP	S	S	S	S	S	S	S	S	S	S	I
36	163668	M	42	Pus	A.baumannii	surgery	IP	S	S	R	R	R	R	R	R	R	R	I
37	184578	M	63	Pus	A.baumannii	surgery	IP	R	R	R	R	R	R	R	S	R	R	I
38	164005	M	45	Pus	A.lwoffii	surgery	IP	R	S	R	R	S	R	R	R	S	S	I
39	162002	M	55	Pus	P.aeruginosa	ortho	IP	S	S	S	S	S	S	S	S	S	S	I
40	163003	M	45	drain tip	A.lwoffii	OG	IP	S	S	S	S	S	S	S	S	S	S	I
41	1159250	F	70	ear swab	A.lwoffii	ENT	OP	S	S	S	S	R	S	S	R	S	S	I
42	163858	M	38	Pus	P.aeruginosa	medicine	IP	S	R	S	S	S	R	S	R	S	S	I
43	162591	M	56	Pus	P.aeruginosa	surgery	I	S	S	S	S	S	S	S	S	S	S	I

							P											
44	165299	M	50	Pus	A.lwoffii	OG	IP	S	S	R	S	S	S	S	R	S	S	I
45	165370	M	64	Pus	A.lwoffii	surgery	IP	S	S	S	S	S	S	S	S	S	S	I
46	1145282	M	65	Pus	P.aeruginosa	surgery	OP	R	R	R	R	R	R	R	R	R	R	I
47	1018352	M	35	Pus	A.baumannii	surgery	IP	S	S	S	S	S	S	S	R	S	S	I
48	165153	M	22	Pus	P.aeruginosa	surgery	IP	R	R	R	R	R	R	R	R	R	R	I
49	165222	F	21	Pus	P.aeruginosa	ortho	IP	S	S	R	R	S	S	S	S	S	S	I
50	164578	M	70	Pus	P.fluorescens	ortho	IP	R	R	S	R	R	R	R	S	R	R	I
51	165216	M	20	Pus	P.aeruginosa	ortho	IP	R	R	R	R	R	R	S	S	R	R	I
52	166534	M	52	Pus	A.baumannii		IP	R	R	S	S	R	R	R	S	R	R	I
53	166187	F	47	Pus	P.aeruginosa	surgery	IP	S	R	S	S	R	S	S	S	S	S	I
54	160581	M	55	Pus	A.lwoffii	OG	IP	S	S	S	S	S	S	S	S	S	S	I
55	164816	F	40	Pus	A.lwoffii	ENT	IP	S	S	S	S	S	S	S	S	S	S	I
56	750283	F	37	Pus	P.aeruginosa	ortho	OP	R	R	S	R	R	R	S	R	S	S	I
57	165956	F	40	Pus	P.fluorescens	surgery	IP	S	S	S	S	S	S	S	S	S	S	I
58	646668	M	65	Pus	P.aeruginosa	OG	OP	R	S	S	S	S	S	S	S	S	S	I
59	1056099	F	22	ear swab	P.aeruginosa	ENT	OP	S	S	S	S	S	S	S	S	S	S	I
60	1149159	F	65	Pus	A.lwoffii	surgery	OP	S	S	S	R	S	S	S	S	S	S	I
61	1180742	M	17	ear swab	P.aeruginosa	ENT	OP	S	R	S	S	S	S	S	S	S	S	I
62	166850	M	35	Pus	A.baumannii	surgery	IP	R	R	R	R	R	R	R	S	R	R	I
63	167617	M	37	Pus	P.aeruginosa	surgery	IP	S	S	S	S	S	S	S	S	S	S	I
64	1183240	M	53	Pus	P.aeruginosa	surgery	OP	S	S	S	S	S	S	S	S	S	S	I
65	168125	F	60	Pus	A.baumannii	burns	IP	R	R	R	R	R	R	R	S	R	R	I
66	1134693	F	36	Pus	A.lwoffii	surgery	OP	S	S	R	R	S	S	S	S	S	S	I

67	167901	M	12	Pus	P.aeruginosa	icu	IP	S	S	S	S	S	S	S	S	S	S	I
68	168127	F	20	Pus	P.fluorescens	OG	IP	S	S	S	S	S	S	S	S	S	S	I
69	1127318	M	34	Pus	A.lwoffii	ortho	OP	S	S	S	S	R	S	S	S	S	S	I
70	167335	M	33	Pus	P.aeruginosa	surgery	IP	S	S	S	S	S	S	S	S	S	S	I
71	168888	F	70	Pus	A.baumannii	surgery	IP	R	R	R	R	R	R	R	S	R	R	I
72	168909	F	32	Pus	P.aeruginosa	burns	IP	R	R	R	R	R	R	R	R	R	R	I
73	1115707	M	29	ear swab	P.aeruginosa	icu	IP	S	S	S	S	S	S	S	S	S	S	I
74	169042	F	45	Pus	A.lwoffii	icu	IP	S	S	S	S	S	S	S	S	S	S	I
75	166738	F	55	Pus	P.aeruginosa	ortho	IP	R	R	R	R	R	R	S	R	S	S	I
76	168927	M	69	Pus	P.aeruginosa	surgery	IP	R	R	R	R	R	R	R	R	S	S	I
77	166692	F	60	Pus	P.fluorescens	OG	IP	S	S	S	S	S	S	S	S	S	S	I
78	169314	M	35	Pus	P.aeruginosa	ortho	IP	R	R	R	R	S	S	S	S	S	S	I
79	169917	F	81	Pus	P.fluorescens	ENT	IP	S	S	S	S	S	S	S	S	S	S	I
80	169938	M	6	Pus	P.aeruginosa	ortho	IP	S	S	S	S	S	S	S	S	S	S	I
81	168120	F	45	sputum	P.aeruginosa	icu	IP	S	S	S	S	S	S	S	S	S	S	I
82	170105	F	27	Pus	P.aeruginosa	surgery	IP	R	R	S	R	R	S	S	S	S	S	I
83	170260	M	55	Pus	Schwanella	surgery	IP	S	S	S	S	S	S	S	S	S	S	I
84	1200235	F	75	Pus	P.aeruginosa	surgery	IP	S	S	S	S	S	S	S	S	S	S	I
85	169405	M	10	ear swab	A.lwoffii	ENT	IP	S	S	S	S	S	S	S	S	S	S	I
86	169042	F	55	Pus	A.baumannii	surgery	IP	R	S	R	R	R	R	R	S	R	R	I
87	170591	M	58	Pus	P.aeruginosa	ortho	IP	R	R	R	R	R	R	R	S	R	R	I
88	170620	M	65	Pus	A.baumannii	ortho	IP	R	R	R	R	R	R	R	S	R	R	I
89	169996	M	70	Pus	P.aeruginosa	ortho	IP	R	S	R	R	R	R	R	S	R	R	I
90	120356	M	50	Pus	P.aeruginosa	surgery	I	S	R	R	R	R	R	R	R	S	S	I

	8						P											
91	17039	M	65	Pus	P.fluorescens	surgery	IP	S	S	R	R	S	R	R	R	R	S	I
92	170929	F	65	Pus	A.baumannii	OG	IP	R	R	S	S	R	R	R	S	S	S	I
93	170337	M	48	Pus	P.aeruginosa	ENT	IP	R	R	R	R	R	R	S	S	S	S	I
94	170926	M	47	sputum	P.aeruginosa	icu	IP	S	S	S	S	S	S	S	S	S	S	I
95	169456	F	18	sputum	P.aeruginosa	icu	IP	S	S	S	S	S	S	S	S	S	S	I
96	171036	M	83	sputum	P.aeruginosa	icu	IP	S	S	S	S	R	S	S	S	S	S	I
97	171215	M	50	sputum	P.aeruginosa	thoracic medicine	OP	S	S	S	S	S	S	S	S	S	S	I
98	170260	F	46	sputum	Flavobacterium	icu	IP	S	S	S	S	R	S	S	S	S	S	D
99	156874	M	51	sputum	P.aeruginosa	thoracic medicine	IP	S	S	S	S	R	S	S	S	S	R	I
100	160794	M	51	sputum	P.aeruginosa	thoracic medicine	OP	S	S	S	S	S	S	S	S	S	S	I
101	162975	F	45	sputum	P.aeruginosa	thoracic medicine	IP	S	S	S	S	S	S	S	S	S	S	I
102	1152917	M	47	sputum	P.aeruginosa	thoracic medicine	OP	S	S	S	S	S	S	S	S	S	S	I
103	163722	M	45	sputum	P.aeruginosa	thoracic medicine	IP	S	S	S	S	S	S	S	S	S	S	I
104	163899	M	68	sputum	Flavobacterium	thoracic medicine	IP	S	S	S	S	S	S	S	S	S	S	I
105	1163339	M	78	sputum	A.lwoffii	thoracic medicine	IP	S	S	S	S	S	S	S	S	S	S	I
106	165367	M	65	sputum	P.aeruginosa	thoracic medicine	IP	S	S	S	S	S	S	S	S	S	S	I
107	1100365	M	15	sputum	P.aeruginosa	thoracic medicine	IP	S	S	S	S	S	S	S	S	S	S	I
108	166644	M	35	sputum	P.aeruginosa	thoracic medicine	IP	R	R	S	S	S	S	S	S	S	S	I
109	165551	M	66	sputum	A.baumannii	thoracic medicine	IP	S	S	S	S	S	S	S	S	S	S	I
110	166971	M	62	sputum	Achromobacter	thoracic medicine	IP	S	S	S	S	S	S	S	S	S	S	I
111	167378	M	65	sputum	P.aeruginosa	thoracic medicine	IP	S	S	S	S	S	S	S	S	S	S	I
1121	167481	M	55	sputum	P.aeruginosa	thoracic medicine	IP	S	S	S	S	S	R	S	R	S	S	I
13	167336	M	47	sputum	A.baumannii	thoracic medicine	IP	S	S	R	S	R	R	R	S	R	R	I

114	167619	F	29	sputum	A.lwoffii	thoracic medicine	IP	S	S	S	S	S	S	S	S	S	S	I
115	167860	M	65	sputum	A.baumannii	thoracic medicine	IP	S	S	S	R	S	R	R	S	R	R	I
116	168350	F	57	sputum	A.baumannii	thoracic medicine	IP	R	R	S	R	R	R	S	S	R	R	I
117	168491	F	55	ET tip	A.baumannii	icu	IP	S	R	R	R	R	R	R	S	R	S	I
118	169514	M	50	sputum	Achromobacter	thoracic medicine	IP	S	S	S	S	S	S	S	S	S	S	I
119	169882	M	33	sputum	P.aeruginosa	thoracic medicine	IP	S	S	S	S	S	S	S	S	S	S	I
120	169939	F	42	Urine	P.aeruginosa	surgery	IP	S	S	S	S	S	S	S	S	S	S	I
121	169992	M	61	Urine	P.aeruginosa	surgery	IP	R	R	R	R	R	R	R	R	R	R	I
122	168151	M	26	Urine	A.baumannii	surgery	IP	R	R	S	S	S	R	R	S	R	R	I
123	171251	F	33	Urine	P.aeruginosa	medicine	IP	S	S	S	S	S	S	S	S	S	S	I
124	171257	M	54	Urine	P.aeruginosa	medicine	IP	S	S	S	S	S	S	S	S	S	S	I
125	159595	M	75	Urine	A.lwoffii	medicine	IP	S	S	S	S	S	S	S	S	S	S	I
126	160133	M	52	Urine	A.baumannii	surgery	IP	R	R	R	R	R	R	R	S	R	R	I
127	160689	F	32	Urine	P.aeruginosa	surgery	OP	S	S	S	S	S	S	S	S	S	S	I
128	164496	M	85	Urine	P.aeruginosa	ortho	IP	S	S	S	S	S	S	S	S	S	R	I
129	164810	F	56	Urine	A.baumannii	medicine	IP	S	S	S	S	S	S	S	S	S	S	I
130	165550	F	56	Urine	P.aeruginosa	surgery	IP	S	S	S	S	R	S	S	S	S	S	I
131	166901	M	65	Blood	A.baumannii	surgery	IP	S	S	S	S	R	R	S	R	S	S	I
132	1178557	M	55	Blood	P.aeruginosa	icu	IP	S	R	R	R	R	S	S	R	S	S	I
133	169400	M	22	Blood	A.baumannii	ortho	IP	S	S	S	R	S	S	S	S	S	S	I
134	157148	F	21	Blood	A.baumannii	medicine	IP	S	S	S	S	S	S	S	S	S	S	I
135	1078704	F	28	Blood	A.baumannii	surgery	IP	R	S	S	S	S	S	S	S	S	S	I
136	625614	M	10	Blood	A.baumannii	paediatric	IP	R	R	R	S	R	S	S	S	S	S	I
13	150813	F	69	Blood	P.aeruginosa	surgery	I	S	S	S	S	S	S	S	S	S	S	I

7							P											
138	155254	M	56	Pus	P.aeruginosa	surgery	IP	S	S	S	S	S	S	S	S	S	S	I
139	165481	F	41	Pus	P.aeruginosa	ortho	IP	S	S	S	S	S	S	S	S	S	S	I
140	724494	F	40	Pus	P.aeruginosa	surgery	IP	S	S	S	S	S	S	S	S	S	R	I
141	1691660	F	69	ear swab	P.aeruginosa	ENT	IP	R	S	R	S	S	S	S	S	S	S	I
142	178296	M	60	Pus	P.aeruginosa	surgery	IP	S	S	S	S	R	R	S	S	S	S	I
143	180656	F	35	Pus	P.aeruginosa	surgery	IP	S	S	S	R	S	S	S	S	S	S	I
144	169606	F	81	Pus	P.stutzeri	surgery	IP	S	S	S	S	S	S	S	R	S	S	I
145	171801	M	52	Pus	A.baumannii	surgery	IP	S	R	R	S	R	S	S	S	R	R	I
146	179443	M	5	Pus	P.aeruginosa	thoracic medicine	IP	S	S	S	S	S	S	S	S	S	S	I
147	1273717	F	60	Blood	A.baumannii	paediatric	IP	S	S	S	S	S	S	S	S	S	S	I
148	179469	F	36	Pus	P.aeruginosa	medicine	OP	S	S	S	S	S	S	S	S	S	S	I
149	180358	M	58	sputum	P.aeruginosa	medicine	IP	S	S	S	R	S	S	S	S	S	S	I
150	179680	M	58	sputum	P.aeruginosa	thoracic medicine	IP	S	S	S	S	S	S	S	S	S	S	I
151	178296	M	50	ET tip	P.aeruginosa	icu	IP	R	R	R	R	R	R	S	S	R	R	I
152	175599	M	50	ET tip	P.aeruginosa	icu	IP	S	S	S	R	S	S	S	S	S	S	I
153	178007	F	76	sputum	P.aeruginosa	surgery	IP	S	S	S	R	S	S	S	S	S	S	I
154	177745	M	72	sputum	A.baumannii	icu	IP	S	S	S	S	S	S	S	S	S	S	I
155	173403	M	65	ET tip	P.aeruginosa	icu	IP	S	S	S	S	S	S	S	S	S	S	I
156	173454	M	75	sputum	P.aeruginosa	icu	IP	S	S	S	S	S	S	S	S	S	S	I
157	176438	M	56	sputum	P.fluorescens	thoracic medicine	IP	S	S	S	S	S	S	S	S	S	S	I
158	176350	M	55	ET tip	P.aeruginosa	icu	IP	S	S	S	S	S	S	S	S	S	S	I
159	176355	M	70	ET tip	P.aeruginosa	icu	IP	S	S	S	R	S	S	R	S	S	S	I
160	180989	M	63	sputum	P.aeruginosa	surgery	IP	S	S	S	S	S	S	S	S	S	S	I

161	181007	M	70	sputum	P.aeruginosa	thoracic medicine	IP	R	R	S	S	R	R	R	S	S	S	I
162	181454	M	55	ET tip	P.aeruginosa	icu	IP	S	S	R	S	S	S	S	S	S	S	I
163	182668	F	23	ET tip	P.aeruginosa	icu	IP	S	S	S	S	S	S	S	S	S	S	I
164	182359	F	62	sputum	P.fluorescens	thoracic medicine	IP	S	S	S	S	S	S	S	S	S	S	I
165	182665	F	26	sputum	P.aeruginosa	icu	IP	S	S	S	S	S	S	S	S	S	S	I
166	183623	M	60	ET tip	P.aeruginosa	icu	IP	S	S	S	R	S	R	S	S	S	S	I
167	176123	M	12	Urine	P.aeruginosa	ortho	IP	S	S	S	S	S	S	S	S	S	S	I
168	178082	M	47	Urine	A.baumannii	surgery	IP	S	S	S	R	R	R	S	S	R	R	I
169	179890	F	38	Urine	P.aeruginosa	OG	IP	S	S	S	R	S	S	S	S	R	R	I
170	179095	M	40	Urine	P.aeruginosa	ortho	IP	R	R	R	R	R	R	R	R	R	R	I
171	180778	F	82	Urine	P.aeruginosa	medicine	IP	S	S	S	S	S	S	S	S	S	S	I
172	178880	M	54	Urine	A.baumannii	surgery	IP	S	S	S	S	S	S	S	S	S	S	I

PROFORMA

CLINICAL DETAILS

NAME:

Sl.No. :

Ip . \ Op. no.

AGE \ SEX:

D.O.A:

ADDRESS:

D.O.D:

OCCUPATION:

PRESENTING COMPLAINTS (WITH ANTIBIOTIC HISTORY):

PAST HISTORY:

Hospitalisation	**Treatment**	**Any invasive procedure**

HISTORY OF ASSOCIATED ILLNESS: Diabetes/HIV/other immunocompromised status

GENERAL PHYSICAL EXAMINATION:

PROVISIONAL DIAGNOSIS:

TREATMENT

INVESTIGATIONS DONE:

A) GENERAL:

B) **MICROBIOLOGY**

Sample and date of collection:

Site and clinical diagnosis:

Culture : **Gram Stain**

NA

MAC --- NLF colonies – TSI -

BAP

GRAMS	**OF**	**10 % LAC**	**Decarboxylation**
PIGMENT	**G**	**Es -**	**Lys**
OXIDASE	**L**		**Arg**
MOTILITY	**S**		**Orn**
INDOLE	**MAL**		
UREASE	**MANN**		
NR TEST	**XYLs**		

Organism isolated -

ANTIBIOGRAM :

Antibiotic	Zone	Interpretation	Antibiotic	Zone	interpretation
CAZ			IMP		
CPM			MER		
AT			DOR		
PTZ					
G			COL		
AK			POL B		
CIP					
LEV			CTX		
NOR			CTR		
			DOX		
			COT		

Patient’s response to treatment :

ANNEXURE II

BIBLIOGRAPHY

1. Winn W Jr, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P, *et al*., editors. Nonfermenting Gram negative bacilli. In: Koneman's Color Atlas and textbook of diagnostic Microbiology, 6th ed. USA: Lippincott Williams and Wilkins Company; 2006. p. 305-91.
2. Malini A, Deepa E.K, Gokul B.N, Prasad S.R. Non-fermenting Gram Negative bacilli infections in a Tertiary care hospital in Kolar, Karnataka. J Lab Physicians 2009; 1(2) : 62-66.
3. Chawla K, Vishwanath S, and Munim F. C. Nonfermenting Gram-negative Bacilli other than *Pseudomonas aeruginosa* and *Acinetobacter* Spp. causing Respiratory Tract Infections in a Tertiary Care Center. J Glob Infect Dis. 2013 Oct-Dec; 5(4): 144–148.
4. Rit K, Nag F, Jyoti Raj K, Maity P K, **"**Prevalence and Susceptibility Profiles of Nonfermentative Gram-negative Bacilli Infection in a Tertiary Care Hospital of Eastern India**"** Indian Journal of Clinical Practice, October 2013; 24(5):451-455.
5. Rahbar M, Mehragan H, Ali Akbari N H, "Prevalence of Drug Resistance in Nonfermenter Gram-Negative Bacilli" Iran J Pathol (2010)5 (2), 90 – 96.

6. KL S , Rao G G, Kukkamalla A M. Prevalence Of Non-fermenters In Urinary Tract Infections In A Tertiary Care Hospital . WebmedCentral MICROBIOLOGY 2011;2(1):WMC001464.
7. Juyal D, Prakash R, Shamanth A, et al., "Prevalence of non‑fermenting gram negative bacilli and their *in vitro* susceptibility pattern in a tertiary care hospital of Uttarakhand: A study from foothills of Himalayas" Saudi Journal for Health Sciences, May-Aug 2013; 2(2):108-112.
8. Hancock RE. Resistance mechanisms in Pseudomonas aeruginosa and other nonfermentative gram-negative bacteria. Clin Infect Dis 1998; 27(1): 93-99.
9. Bokaeian M, Amini BN, Sahi Z, Saeidi S. Antibacterial activities of Myrtus communis L extract against multi-drug resistant Klebsiella pneumonia and Pseudomonas aeruginosa. Inter J Biosciences. 2013; 4(9): 254-259.
10. Bahar M. A., Jamali S. and Samadikuchaksaraei A. Imipenem- resistant Pseudomonas aeruginosa strains carry metallo-b-lactamase gene blaVIM in a level I Iranian burn hospital. Burn elsiver journal, 2010; 36: 826-830.
11. Vasundhara Devi P, Sreenivasalu Reddy P, Sindhura John M. Prevalence of Metallo--Lactamases Producing Pseudomonas aeruginosa among the Clinical isolates: A study from tertiary care hospital. Inter J Current Microbiol Applied Sci 2015; 4(4): 955-961.
12. Shahcheraghi F, Abbasalipour M, Feizabadi MM, Ebrahimipour GH, Akbari NK. Isolation and genetic characterization of metallo-β-lactamase and carbapenamase producing strains of *Acinetobacter baumannii*from patients at Tehran hospitals. Iran J Microbiol 2011; 3(2): 68-74.

13. Hirakata Y, Yamaguchi T, Nakano M. Clinical and Bacteriological Characteristics of IMP-Type Metallo-b-Lactamase–Producing *Pseudomonas aeruginosa* . Clinical Infectious Diseases 2003; 37:26–32.
14. Hemalatha V, Uma Sekar. Detection of metallo betalactamase producing *Pseudomonas aeruginosa* in hospitalized patients.Indian J Med Res, August 2005;148-152.
15. Karthika U et al., 2009 Karthika R U, Rao R S, Sahoo S, Shashikala P. Phenotypic and genotypic assays for detecting the prevalence of metallo-b-lactamases in clinical isolates of Acinetobacter baumannii from a South Indian tertiary care hospital. J Med Microbiol 2009; 58: 430–435.
16. Marra AR, Pereira CA, Gales AC, Menezes LC, Cal RG, de Souza JM, Edmond MB, Faro C, Wey SB. Blood stream infections with Metallo-beta- lactamase producing Pseudomonas aeruginosa: epidemiology, microbiology and clinical outcomes. Antimicrob Agents Chemother 2006, 50:388-390.
17. Mandell Gerald L, Bennet John E. Dolin Raphael Principles and Practice of Infectious Diseases. 7th edition, 2010; Churchill Livingstone Elsevier; 2835- 2964.
18. Collier L, Ballows A, Sussman M. Topley and Wilson's Microbiology and Microbial Infections, Volume 2 , 18th edition, ASM press.
19. Vandamme P, Bernardet J, Segers P, Kersters K, Holmes B. Notes: New Perspectives in the Classification of the Flavobacteria: Description of Chryseobacterium gen. nov., Bergeyella gen. nov., and Empedobacter nom. rev. International Journal of Systematic Bacteriology. 1994;44(4):827-831.

20. Lister PD, Wolter DJ, Hanson ND. Antibacterial-Resistant *Pseudomonas aeruginosa*: Clinical Impact and Complex Regulation of Chromosomally Encoded Resistance Mechanisms. Clin Microbiol Rev 2009; 22(4): 582-610.

21. Fournier PE, Vallenet D, Barbe V, Audic S, Ogata H, Poirel L, Richet H, Robert C, Mangenot S, Abergel C, Nordmann P, Weissenbach J, Raoult D. Comparative Genomics of Multidrug Resistance in *Acinetobacter baumanni*. *Plos Genet* 2006; 2(1): e7.

22. Lowbury, Kidson ELJ, Lilly HA. Sensitivity of *Pseudomonas aeruginosa* to antibiotics: emergence of strains highly resistant to carbenicillin. Lancet 1969; 2: 448-452.

23. Rajendra D, Ramana BV, Chaudhury A. Spectrum of non fermenting gram negative bacilli infection (excluding pseudomonads) in a tertiary care hospital. Int J Biol Med Res. 2012; 3(3): 1892-1894.

24. Brooks Geo F, Carroll Karen C, Butel Janet S, Morse Stephen A, Mietzner Timothy A. Pseudomonads, Acinetobacters & uncommon Gram negative bacilli Jawetz, Melnick and Adelberg's Medical Microbiology, 25th edition, 2001, McGraw Hill; 227-233.

25. Anzai Y, Kim H, Park JY, Wakabayashi H, Oyaizu H. Phylogenetic affiliation of the pseudomonads based on 16S rRNA sequence. Int J Syst Evol Microbiol. 2000 Jul; 50(4):1563-89

26. Gilardi GL. Pseudomonas and related genera. In:cBalows A, ed. Manual of Clinical Microbiology.Ed.5. Washington DC:American society for Microbiology,1991:429-441.

27. Palleroni NJ.Family I.Pseudomonaceae.In:Kreig NR, Holt JG,eds.Bergy,s Manual of Systemic Bacteriology.Vol !. Baltimore: Williams & Wilkins, 1984:141-219.

28. King EO, Ward MK, Raney DE. Two simple media for the demonstration of pyocyanin and fluorescein. J Lab Clin Med 1954; 44: 301-307

29. Wong V, Levi K, Baddal B, Turton J, Boswell TC. Spread of Pseudomonas fluorescens Due to Contaminated Drinking Water in a Bone Marrow TransplantUnit. J Clin Microbiol. 2011 June; 49(6): 2093–2096.

30. Silva GA, Almeida EA. Production of Yellow-Green Fluorescent Pigment by Pseudomonas fluorescens. Braz. arch. biol. technol.2006; 49 (3): 411-19.

31. Govan JRW. Pseudomonas, Stenotrophomonas, Burkholderia.Collee JG,Fraser AG,Marimion,Simmons A,editors. Practical Medical Microbiology.14th edition, India: Churchchill Livingstone 2006; 448-461.

32. Bodey GP, Bolivar R, Fainstein V, et al. Infections caused by Psudomonas aeruginosa. Rev Infect Dis 1983;5:279-313

33. Strateva T, Mitov I "Contribution of an arsenal of virulence factors to pathogenesis of Pseudomonas aeruginosa infections" Ann Microbiol2011; 61(4):717-73

34. Ben Haj Khalifa A, Moissenet D, Vu Thien H, Khedher M. "Virulence factors in Pseudomonas aeruginosa: mechanisms and modes of regulation" Ann Biol Clin(Paris). 2011 Jul-Aug;69(4):393-403.

35. Ananthnarayan R, Paniker CKJ, “Pseudomonas” Textbook of Microbiology, 8thedition, 2009, University Press; 315-318.
36. Gales AC,Jones RN, Turnidge J, et al. Characterization of P.aeruginosa isolates: Occurance rates, antimicrobial susceptibility pattern, molecular typing in the global SENTRY antimicrobial surveillance programme, 1997-1999. Clin Infec Dis 32(suppl 2): 2001;S146-S155
37. Livermore, DM: Multiple mechanisms of antimicrobial resistance in Psudomonas aruginosa: our worst nightmare? Clin Infect Dis 2002;34:634-644.
38. Benjamin S. Thomas, Koh Okamoto, MD, Matthew J. Bankowski, Todd B. Seto. A Lethal Case of *Pseudomonas putida* Bacteremia due to SoftTissue Infection**.** Infect Dis Clin Pract 2013; 21(3): 147–213.
39. Murray Patrick R, Baron Ellen Jo, Jorgensen James H, Landry Marie Louise, Manual of clinical Microbiology, Vol I, 8th edition, ASM Press, 2003; 734- 834.
40. Lalucat J, Bennasar A, Bosch R, García-Valdés E, Palleroni NJ. “Biology of Pseudomonas stutzeri” Microbiol Mol Biol Rev. 2006 June; 70(2): 510–547.
41. Aragone MR, Maurizi DM, Clara LO, Navarro Estrada JL, Ascione A. Pseudomonas mendocina, an environmental bacterium isolated from a patient with human infective endocarditis. J Clin Microbiol. 1992 June; 30(6): 1583–1584.
42. Nseir W, Taha H, Abid A, Khateeb J. “Pseudomonas mendocina sepsis in a healthy man.”Isr Med Assoc J. 2011 Jun;13(6):375-6.
43. Potvliege C, Dejaegher BC, Hansen W. Flavobacterium multivorum septicemia in hemodialysed patient. J Clin Microbiol 1984; 9: 568-569.

44. Valenstein P, Bardy GH, Cox CC, Zwadyk P. Pseudomonas alcaligenes endocarditis. Am J Clin Pathol 1983; 79(2): 245-247.

45. Chihab W, Alaoui AS, Amar M. Chryseomonas luteola identified as the source of serious infections in a Moroccan University Hospital. J Clin Microbiol 2004;42:1817-1839.

46. Rahav G, Simhon A, Mattan Y, et al. Infections with Chryseomonas luteola (CDC group Ve-1) and Flavimonas oryzihabitans (CDC group Ve-2). Medicine (Baltimore)1995;4:83-88.

47. Esteban J, Martin J, Ortiz A, et al. Pseudomonas oryzihabitans peritonitis in a patient on continuous ambulatory peritoneal dialysis. Clin Microbiol Infect 2002;8: 607-608.

48. Thomassen MJ, Demko CA, Klinger JD et al. Pseudomonas cepacia colonization among patients with cystic fibrosis: a new opportunist. Am Rev Respir Dis 1985;131:791-796

49. Gilligan BH. Microbiology of airway disase in patients with cystic fibrosis. Clin Microbiol Rev 1991;4:35-51

50. Welch DF, Muszynski MJ, Pai CH et al. Selective and differential medium for recovery of Pseudomonas cepacia from respiratory tracts of patients with cystic fibrosis. J Clin microbiol 1987;25:1730-1734.

51. Henry D, Campbell M, McGimpsey C, et al. Comparision of isolation media for recovery of Burkholderia cepacia complex from respiratory secretions of patients with cystic fibrosis. J Clin Microbiol 1999;37:1004-1007

52. Premjit A, Wirongrong C, Vanaporn W, Yuvadee M, Rattanaphone P, Bart JC, Paul NN. Accuracy of Burkholderia pseudomallei identification using the API 20NE system and Latex agglutination test. J Clin Microbiol 2007; 45(11): 3774.

53. Peter H Gilligan. Infections in patients with Cystic Fibrosis. Diagnostic Microbiol Update 2004; 34(2): 197-217.

54. Ashdown LR. An improved screening technique for isolation of Pseudomonas pseudomallei from clinical specimens. Pathology 1979; 11(2): 293-297.

55. Reina J, Bassa A, Llompart I, Portela D, et al. Infections with Pseudomonas paucimobilis: report of four cases and review. Rev Infect Dis 1991;13:1072-1076.

56. Pagani L, Lang A, Vedovelli C, et al. Soft tissue infection and bacterimia caused by Shewanella putrefaciens. J Clin Microbiol 2003;41:2240-2241

57. Aisenberg G, Rolston KV, Safdar A. Bacteremia caused by *Achromobacter* and *Alcaligenes* species in 46 patients with cancer (1989–2003). Oncology and Radiother 2004; 101(9): 2134-2140.

58. Kiska DL, Gilligan PH. Pseudomonas. Murray PR,Baron EJ,Jorgensen JH,Pfaller MA,Yolken RH,editors. Manual of Clinical Microbiology. 8th edition vol I, Washington DC: ASM Press 2003; 719-728.

59. Ceyhan M, Celik M. Elizabethkingia meningosepticum (Chryseobacterium meningosepticum) Infections in Children. International Journal of Pediatrics. 2011;2011:1-7.

60. Dias M, Prashant K, Pai R, Scaria B. Chryseobacterium meningosepticum bacteremia in diabetic nephropathy patient on hemodialysis. Indian J Nephrol. 2010;20(4):203-204.

61. Sofia C, Angela R, Luminita SL, Raluca F, Luliana T. Cultural And Biochemical Characteristics Of Acinetobacter Spp. Strains Isolated From Hospital Units. J Prev Med 2004; 12(3-4): 35-42.

62. Mindolli P, Salmani M, G V, A.R H. Identification And Speciation Of Acinetobacter And Their Antimicrobial Susceptibility Testing. Al Ameen J Med Sc. 2010;3(4):345 - 349.

63. Prashanth K, badrinath S. Simplified phenotypic tests for identification of Acinetobacter spp. and their antimicrobial susceptibility status. Journal of Medical Microbiology. 2000;49(9):773-778.

64. Oteo J, Campos J, Baquero F. Antibiotic resistance in 1962 invasive isolates of *Escherichia coli* in 27 Spanish hospitals participating in the European antimicrobial resistance surveillance system. *Journal of Antimicrobial Chemotherapy, 2002;* 50: 945-952.

65. Courvalin P. Evasion of antibiotic action by bacteria. *Journal of Antimicrobial Chemotherapy*, 1996; 37: 855-869

66. Meletis G, Exindari M, Vavatsi N, Sofianou D, Diza E " Mechanisms responsible for the emergence of carbapenem resistance in Pseudomonas aeruginosa." Hippokratia. 2012;16(4):303-7.

67. Horii T, Muramatsu H, Morita M, Maekawa M. Characterization of *Pseudomonas aeruginosa* isolates from patients with urinary tract infections during antibiotic therapy. Microb Drug Resist 2003; 9:223–9.

68. Gotoh N, Nagino K, Wada K, Tsujimoto H, Nishino T. *Burkholderia* (formerly *Pseudomonas*) *cepacia* porin is an oligomer composed of two component proteins. Microbiol **1994**;10:3285–91.

69. Sato K, Nakae T. Outer membrane permeability of *Acinetobacter calcoace ticus* and its implication in antibiotic resistance. J Antimicrob Chem ther **1991**;28:35–45.

70. Yamazaki E, Ishii J, Sato K, Nakae T. The barrier function of the outer membrane of Pseudomonas maltophilia in the diffusion of saccharides and beta-lactam antibiotics. FEMS Microbiol Lett 1989; 51(1): 85-88.

71. Poole K, Krebes K, McNally C, Neshat S. Multiple antibiotic resistance in Pseudomonas aeruginosa: evidence for involvement of an efflux operon. J Bacteriol 1993; 175(22): 7363-7372.

72. Bou G, Martinez-Beltran J. Cloning, nucleotide sequencing, and analysis of the gene encoding an AmpC b-lactamase in *Acinetobacterbaumannii*. Antimicrob Agents Chemother **2000**; 44:428–32.

73. Maragakis LL, Perl TM. Acinetobacter baumannii: epidemiology, antimicrobial resistance, and treatment options. Clin Infect Dis. 2008; 46: 1254–63.

74. *Poirel L, Lebessi E, Castro M, Fevre C, Foustoukou M, Nordmann P.* Nosocomial Outbreak of Extended-Spectrum β-Lactamase SHV-5-Producing Isolates

of *Pseudomonas aeruginosa* in Athens, Greece. Antimicrobial Agents Chemother *2004; 48(6): 2277-2279.*

75. *Queenan AM, Bush K.* Carbapenemases: the versatile beta-lactamases. Clin Microbiol Rev *2007; 20(3): 440-458.*

76. Nordmann P, Poirel L. Emerging carbapenemases in Gram-negative aerobes. Clin Microbiol Infect 2002; 8(6): 321-331.

77. Osano E, Arakawa Y, Wacharotayankun R, Ohta M, Horii T, Ito H, et al. Molecular characterization of an enterobacterial metallo-β-lactamases found in a clinical isolates of *Serratia marcescens* that shows imipenem resistance. Antimicrob Agents Chemother 1994;38:71–8

78. Lauretti L, Riccio ML, Mazzariol A, Cornaglia G, Amicosante G, Fontana R, et al. Cloning and characterization of *bla*VIM, a new integron-borne metallo-β-lactamase gene from a *Pseudomonas aeruginosa* clinical isolate. Antimicrob Agents Chemother 1999; 43:1584–90.

79. L. Poirel, M.Magalhaes, M. Lopes, and P. Nordmann, "Molecular analysis of metallo-beta-lactamase gene bla (SPM-1) surrounding sequences from disseminated *Pseudomonas aeruginosa* isolates in Recife, Brazil," *Antimicrobial Agents and Chemotherapy*, vol. 48, no. 4, pp. 1406–1409, 2004.

80. Lee JH, Lequette Y, Greenberg EP. Activity of purified QscR, a *Pseudomonas aeruginosa* orphan quorum-sensing transcription factor. Molecular Microbiology 2006; 59(2): 602-609.

81. Mouneimne H, Robert J, Jarlier V, Cambau E. Type II Topoisomerase Mutations in Ciprofloxacin-Resistant Strains of *Pseudomonas aeruginosa.* Antimicrob Agents Chemother 1999; 43(1): 62-66.

82. Bonomo R, Szabo D. Mechanisms of Multidrug Resistance in Acinetobacter Species and Pseudomonas aeruginosa. Clinical Infectious Diseases. 2006;43:49–56.

83. Manchanda V, Sinha S, Singh N. Multidrug resistant Acinetobacter. J Global Infect Dis. 2010; 2(3): 291.

84. Pandya NP, Prajapati SB, Mehta SJ, Kikani KM, Joshi KP. Evaluation of various methods for detection of metallo-β-lactamase (MBL) production in gram negative bacilli. Int J Biol Med Res 2011; 2(3): 775-777.

85. Umadevi S, Joseph NM, Kumari K, Easow JM, Kumar S, Stephen S, Srirangaraj S, Raj S. Detection of extended spectrum beta lactamases, ampc beta lactamases and metallobetalactamases in clinical isolates of ceftazidime resistant *Pseudomonas Aeruginosa.* Braz J Microbiol 2011; 42(4): 1284- 1288.

86. Picao RC, Andrade SS, Nicoletti AG, Campana EH, Moraes GC, Mendes RE, et al. Metallo-ß-Lactamase Detection: Comparative Evaluation of Double Disk Synergy versus Combined Disk Tests for IMP-, GIM-, SIM-, SPM-, or VIMProducing Isolates. J Clin Microbiol. 2008;46(6):2028-37.

87. Galani I, Rekatsina PD, Hatzaki D, Plachouras D, Souli M, Giamarellou H. Evaluation of different laboratory tests for the detection of metallo-ßlactamase production in Enterobacteriaceae. J Antimicrob Chemother. 2008:61;548-53

88. Yong D, Lee K, Yum J, Shin H, Rossolini G, Chong Y. Imipenem-EDTA Disk Method for Differentiation of Metallo-beta Lactamase-Producing Clinical Isolates of Pseudomonas spp. and Acinetobacter spp. Journal of clinical microbiology. 2002;40(10):3798–3801.

89. Lee K, Lim YS, Yong D, Yum JH, Chong Y. Evaluation of the Hodge test and the imipenem–EDTA double-disc synergy test for differentiating metallo-ßlactamase-producing isolates of Pseudomonas spp. and Acinetobacter spp. J Clin Microbiol 2003;41:4623-9.

90. Behera B, Mathur P, Das A, Kapil A, Sharma V. An evaluation of four different phenotypic techniques for detection of metallo-beta-lactamase producing Pseudomonas aeruginosa. Indian J Med Microbiol 2008; 26(3): 233-237.

91. National Committee for Clinical Laboratory Standards. Performance Standards for antimicrobial susceptibility testing: Twenty fourth informational supplement. NCCLS document M100-S24, Vol.34 No. 1. NCCLS, Wayne, PA: 2014.

92. Segal H. Use of Etest MBL strips for the detection of carbapenemases in Acinetobacter baumannii. J Antimicrobial Chemotherapy. 2005; 56(3): 598-598.

93. Khosravi Y, Tee Tay S, Vadivelu J. Metallo-β-lactamase–producing imipenem-resistant Pseudomonas aeruginosa clinical isolates in a university teaching hospital in Malaysia: detection of IMP-7 and first identification of IMP-4, VIM-2, and VIM-11. Diagnostic Microbiology and Infect Dis. 2010; 67(3): 294-296.

94. Tijet N, Boyd D, Patel S, Mulvey M, Melano R. Evaluation of the Carba NP Test for Rapid Detection of Carbapenemase-Producing Enterobacteriaceae and Pseudomonas aeruginosa. Antimicrobial Agents and Chemotherapy. 2013;57(9):4578-4580.

95. Poirel L, Nordmann P. Rapid tests for detection of carbapenemase producers in P. aeruginosa; what do we really need?. Enfermedades Infecciosas y Microbiología Clínica. 2014;32(10):623-624.

96. Nordmann P, Poirel L, Dortet L. Rapid Detection of Carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis. 2012;18(9):1503-1507.

97. Krueger C, Sheikh W. A new selective medium for isolating Pseudomonas spp. from water. Appl Environ Microbiol. 1887;53(4):895–897.

98. Jawad A, Hawkey P, Heritage J, Snelling A. Description of Leeds Acinetobacter Medium, a new selective and differential medium for isolation of clinically important Acinetobacter spp., and comparison with Herellea agar and Holton's agar. J Clin Microbiol. 1994;32:2353–2358.

99. Walsh TR, Bolmstrom A, Qwarnstrom A, Gales A. Evaluation of a new E test for detecting metallo-β-lacatamases in routine clinical testing. J Clin Microbiol 2002;40:2755-9.

100. Meenakumari S, Verma S, Absar A, Chaudhary A. Antimicrobial susceptibility pattern of clinical isolates of Pseudomonas aeruginosa in an Indian cardiac hospital. International journal of Engineering Science and Technology. 2011;3(9):7117-7123.

101. Anil C, Shahid R. Antimicrobial susceptibility patterns of Pseudomonas aeruginosa clinical isolates at a tertiary care hospital in Kathmandu, Nepal. Asian J Pharm Clin Res. 2013;6(3):235-238.

102. Rashid A, Chowdhury A, Rahman S, Begum S, Muazzam N. Infections by Pseudomonas aeruginosa and antibiotic resistance pattern of the isolates from Dhaka medical college hospital'. Bangladesh J Med Microbiol. 2007;1(2):48-51.

103. Mohanasoundaram KM. The antibiotic resistance pattern in the clinical isolates of *Pseudomonas aeruginosa* in a tertairy care hospital; 2008-2010 (A 3 year study). J Clin Diagn Res 2011;5(3):491-94.

104. Murugan, S., Bakkiya Lakshmi, R., Uma Devi, P. and Mani, K. (2010). 'Prevalance and antimicroial susceptibility pattern of Metallo beta lactamase producing Pseudomonas aeruginosa in Diabetic foot infection'. International Journal of Microbiological Research, 1(3), pp.123-128.

105. Tripathi P, Banerjee G, Saxena S, Gupta M, Ramteke P. Antibiotic resistance pattern of Pseudomons aeruginosa isolated from patients of lower respiratory tract infection. African journal of Microbiology Research. 2011;5(19):2955-2959.

106. Gokale, S. and Metgud, S. (2012). Journal f Pharmaceutical and biomedical sciences. Characterization and antibiotic sensitivity pattern of nonfermenting gramnegative bacilli from various clinical specimens in a tertiary care hospital, belgaum', 17(17), pp.1-5.

107. Yousefi, S., Farajnia, S., Nahaei, M., Akhi, M., Ghotaslou, R., Soroush, M., Naghili, B. and Jazani, N. (2010). Detection of metallo-β-lactamase–encoding genes among clinical isolates of Pseudomonas aeruginosa in northwest of Iran. Diagnostic Microbiology and Infectious Disease, 68(3), pp.322-325.

108. Pena, A., Donato, A., Alves, A., Leitao, R. and Cardoso, O. (2008). Detection of Pseudomonas aeruginosa producing metallo-β-lactamase VIM-2 in a central hospital from Portugal. Eur J Clin Microbiol Infect Dis, 27(12), pp.1269-1271.

109. Aktaş, Z. and Kayacan, Ç. (2008). Investigation of metallo-beta-lactamase producing strains of Pseudomonas aeruginosa and Acinetobacter baumannii by E-test, disk synergy and PCR. Scandinavian Journal of Infectious Diseases, 40(4), pp.320-325.

110. Chu, Y., Cheung, T., Ngan, J. and Kam, K. (2005). EDTA susceptibility leading to false detection of metallo-β-lactamase in Pseudomonas aeruginosa by Etest and an imipenem–EDTA disk method. International Journal of Antimicrobial Agents, 26(4), pp.340-341

111. Behera, B., Mathur, P., Das, A., Kapil, A. and Sharma, V. (2008). An evaluation of four different phenotypic techniques for detection of metallo beta lactamase producing Pseudomonas aeruginosa. Indian journal of medical microbiology, 26(3), pp.233-237.

112. Fallah F, Borhan R, Hashem A. Detection of bla(IMP) and bla(VIM) metallo-β-lactamases genes among Pseudomonas aeruginosa strains. Int J Burn Trauma. 2013;3(2):122-124.

113. Aghamiri S, Amirmozafari N, Fallah Mehrabadi J, Fouladtan B, Samadi Kafil H. Antibiotic Resistance Pattern and Evaluation of Metallo-Beta Lactamase Genes Including bla-IMP and bla-VIM Types in Pseudomonas aeruginosa Isolated from Patients in Tehran Hospitals. ISRN Microbiology. 2014;2014:1-6.

114. Fazzeli H, Akbari R, Moghim Sh, Narimani T, Arabestani MR, Ghoddousi AR. Pseudomonas aeruginosa infections in patients, hospital means, and personnel's specimens. J Res Med Sci 2012; 17(4): 332-7.

115. Trautmann, M., Michalsky, T., Wiedeck, H., Radosavljevic, V. and Ruhnke, M. (2001). Tap Water Colonization With Pseudomonas aeruginosa in a Surgical Intensive Care Unit (ICU) and Relation to Pseudomonas Infections of ICU Patients. Infection Control and Hospital Epidemiology, 22(1), pp.49-52.

116. Bert, F., Maubec, E., Bruneau, B., Berry, P. and Lambert-Zechovsky, N. (1998). Multi-resistant Pseudomonas aeruginosa outbreak associated with contaminated tap water in a neurosurgery intensive care unit. Journal of Hospital Infection, 39(1), pp.53-62.

117. Buttery, J., alabaster, S., Heine, R., Scott, S., Crutchfield, R. And Garland, S. (1998). Multiresistant Pseudomonas aeruginosa outbreak in a pediatric oncology ward related to bath toys. The Pediatric Infectious Disease Journal, 17(6), pp.509-513.

118. Pina, P., Guezenec, P., Guyot, L., Ghnassia, J. and Allouch, P. (1998). An Acinetobacter baumannii outbreak at the versailles hospital centre. Pathol Biol, 46, pp.385-394.